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Redox sites of NMDA receptors can modulate epileptiform activity in hippocampal slices from kainic acid-treated rats

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Abstract

Using an animal model of temporal lobe epilepsy, the kainic acid lesioned rat hippocampus, we have evaluated the possibility of modulating glutamate *N*-methyl-D-aspartate (NMDA) receptor-dependent evoked epileptiform activity through the manipulation of NMDA receptor redox sites. Epileptiform activity was recorded extracellularly from hippocampal slices, in the stratum pyramidale of the CA1 area, and the effects of the oxidizing reagent 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and the reducing agent Tris(2-carboxy ethyl)phosphine (TCEP) on these responses were quantified. Epileptiform activity was substantially reduced in the presence of DTNB but was fully reinstated with the application of TCEP. The effects of both drugs persisted even after wash. Epileptiform activity was totally abolished in the presence of the NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid. These results suggest that epileptiform activity can be controlled by manipulation of the redox sites of NMDA receptors and raise the possibility of developing new anticonvulsant drugs which do not fully block NMDA receptor-mediated synaptic transmission.

Keywords: Temporal lobe epilepsy; *N*-Methyl-D-aspartate receptors; Redox potential; Anticonvulsant drug; CA1; Hippocampus

In various models of temporal lobe epilepsy (TLE) as well as in human tissue, evoked epileptiform discharges depend upon the activation of a specific glutamate receptor, the *N*-methyl-D-aspartate (NMDA) receptor [2,3,7, 14]. The activation of NMDA receptors underlie various neuronal processes including α-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA) receptor-mediated synaptic plasticity. Employment of NMDA receptors antagonists to control epileptiform discharges is thus precluded by their undesirable side-effects. As a consequence, new strategies to control the over expression of NMDA receptor-mediated activity in epileptic tissue need to be found. This report focuses on the redox sites of NMDA receptors as candidates for the modulation of NMDA-dependent epileptiform activity. Indeed, the oxidizing reagent 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) is known to irreversibly decrease NMDA receptor-mediated responses [1], and to prevent NMDA receptor-mediated synaptic plasticity, without affecting NMDA-dependent AMPA receptor-mediated synaptic plasticity [4,8,9].

Following a unilateral intracerebroventricular injection of kainic acid (KA) in the rat, most of the ipsilateral CA3 pyramidal cells degenerate and their Schaffer collaterals disappear. This deafferentation triggers a reactive synaptogenesis in the proximal dendrites of the CA1 pyramidal cells [16] which seems to originate from axon collaterals of CA1 pyramidal cells [15]. Seven days following the lesion, CA1 pyramidal cells are characterized by NMDA-dependent epileptiform activity, while GABAergic inhibition does not seem to be fully functional [18]. In the present study, we have evaluated the possibility to modulate evoked epileptiform activity through the manipulation of the redox sites of NMDA receptors. In this aim, the effects of DTNB and the reducing agent Tris(2-carboxyethyl)phosphine (TCEP) were tested on the evoked epileptiform responses recorded in the pyramidal cell layer of the CA1 area of the KA-lesioned rat hippocampus.

Kainic acid lesions were performed on male Wistar albino rats (180 g) following established procedures [11].

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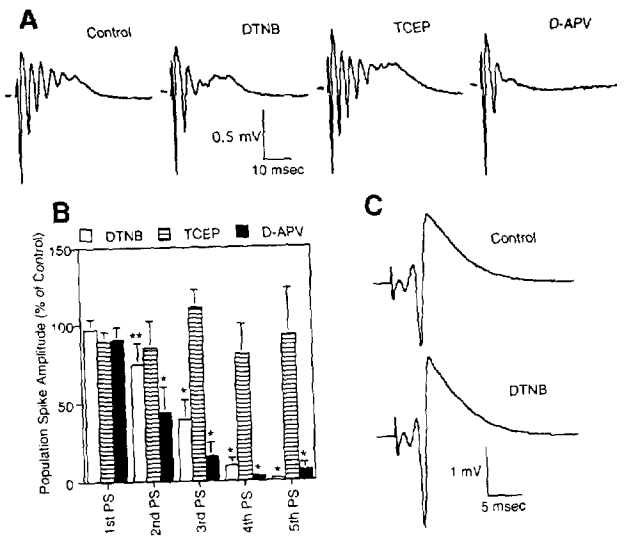

Fig. 1. DTNB irreversibly decreased evoked epileptiform activity in the CA1 area of KA lesioned rat hippocampus. (A) Typical epileptiform responses recorded, under control conditions (first trace) and after exposure to DTNB, TCEP and D-APV. Stimulation artifacts have been erased from all traces for clarity purposes. Traces and all measurements for DTNB and TCEP were obtained after wash out of the drugs. DTNB considerably reduced the late part of the evoked epileptiform activity; TCEP reinstated the epileptiform discharge back to control levels while D-APV abolished it. (B) Histogram of pooled data ($n = 10$) shows the effects of DTNB and D-APV on the mean amplitude of the first five population spikes. Population spike amplitudes have been normalized as a percentage of control (prior to DTNB treatment). DTNB and D-APV had no significant effect on the first population spike whereas they significantly reduced the other population spikes. $^{**}P < 0.05$; $^{*}P < 0.001$. (C) DTNB produced an increase of the population spike amplitude in non-lesioned tissue.

Rats were anaesthetized with chloral hydrate 7% (1 ml, intraperitoneal) and placed in a stereotaxic frame. KA (0.5 μg) in a volume of 0.5 μl of phosphate buffer (pH 7.4) was then infused into the lateral ventricle over a 30 min period. At post-operative day 7, animals were anaesthetized and intracardially perfused with 100 ml of oxygenated ice-cold modified artificial cerebrospinal fluid (ACSF; pH 7.4). Both hippocampi were removed and sliced (450 μm) using a McIlwain tissue chopper. Slices were incubated in oxygenated ACSF for at least 1 h at room temperature before recording and then placed in a submerged in vitro slice chamber. The medium was aerated with 95% O_2 and 5% CO_2. The ACSF was composed of (in mM) NaCl, 124.0; KCl, 3.0; KH_2PO_4, 1.25; $NaHCO_3$, 26; $MgSO_4 \cdot 7H_2O$, 1.3; $CaCl_2$, 2.0 and D-glucose, 10. Temperature in the recording chamber was maintained at 30–32°C. TCEP, DTNB and D-2-amino-5-phosphonovaleric acid (D-APV) were obtained from Pierce, Calbiochem and Tocris, respectively.

Extracellular population spikes were recorded in the slice preparation using 5–10 MΩ resistance glass microelectrodes filled with ACSF. Bipolar twisted-wire stimulation electrodes were placed in the stratum radiatum of CA1 area proximally to stratum pyramidale and 0.5 mm away from the recording electrode. Stimuli (40 μs pulses, 0.5–15 μA) were delivered at 30 s intervals (0.03 Hz). Responses in hippocampi ipsilateral to the lesion were selected if they showed an evoked epileptiform response composed of at least three population spikes. Control experiments were performed in either contralateral slices or in slices of non-lesioned animals after assessing the presence of paired pulse inhibition. Signals were fed to a WPI amplifier. All data were digitized (10 kHz) with a Labmaster interface card to a personal computer and analyzed with Acquis1 program (G. Sadoc, DIPSI, France). Control responses were measured after obtaining a stable baseline (10 min after the start of a stable response, following a 30 min rest in the recording chamber). DTNB (200 μM) or TCEP (200 μM) were applied for 15 min and then washed for 30 min. Evoked responses were measured at the end of the washing procedure. D-APV (50 μM) was applied for 10 min and evoked responses were measured at the end of the drug treatment. Parameters were compared with a Student's t-test for paired samples, for evaluation of differences between means. All values were expressed as mean ± SEM.

Ipsilateral to the lesion, test stimuli in the stratum radiatum evoked epileptiform discharges (Figs. 1A and 2A). The mean amplitude of the first three population spikes composing the epileptiform discharge were 2.1 ± 0.2 mV, 0.8 ± 0.1 mV and 0.16 ± 0.04 mV, respectively ($n = 16$). As in the following, n refers to the number of experiments (one experiment/slice/animal). An example of DTNB treatment is shown in Fig. 1A. Pooled

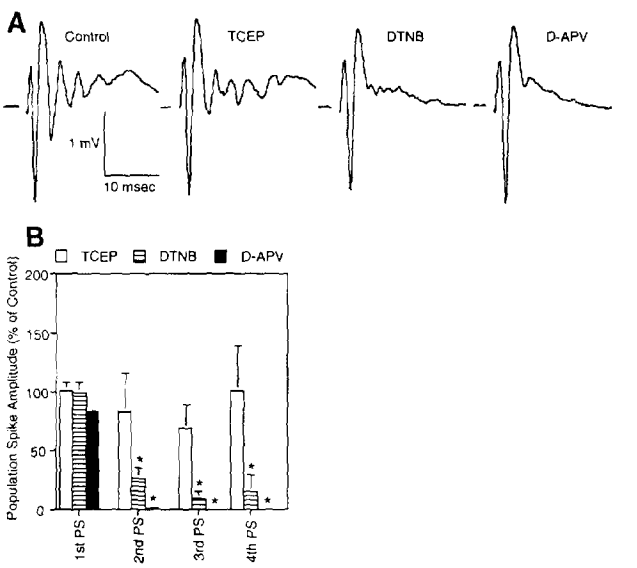

Fig. 2. Effect of TCEP treatment on evoked epileptiform activity. (A) TCEP had no effect on the evoked response apart from desynchronizing the late part of the epileptiform discharge. DTNB and D-APV abolished the late part of the response. (B) Histogram shows the effects of TCEP, DTNB and D-APV on the mean amplitude of the first four population spikes in the KA-lesioned rat hippocampus ($n = 6$). DTNB, TCEP and D-APV had no significant effect on the first population spike. TCEP had no significant effect on the late part of the response whereas DTNB and D-APV abolished it. $^{*}P < 0.001$.

data (Fig. 1B; $n = 10$) show that DTNB did not modify the amplitude of the first population spike (99 ± 7% of control) whereas the second (75 ± 13%), third (39 ± 12%), fourth (9 ± 4%) and fifth (2 ± 2%) population spikes were decreased. Thus, DTNB nearly abolished evoked epileptiform activity. Subsequent treatment with TCEP did not significantly modify the amplitude of the first population spike (90 ± 6% of control) whereas the second (85 ± 17%), third (110 ± 11%) and fourth (81 ± 19%) population spikes returned to control levels. Thus, TCEP reinstated epileptiform activity. Finally, following application of D-APV, the first population spike remained unchanged (92 ± 8% of control) whereas the remaining population spikes were reduced, 44 ± 16% for the second, 15 ± 9% for the third, and the subsequent ones were abolished. In contrast, in a non-lesioned animal slice (Fig. 1C) DTNB increased the population spike (131 ± 10%, $n = 5$, $P < 0.0001$).

These data suggest that pharmacological manipulations of the redox sites directly controls evoked epileptiform activity. Following the KA lesion, the redox state of NMDA receptors is not known. As a first approach to this issue we have applied TCEP before any other drug and investigated its effect on the NMDA-dependent, late part of the epileptiform discharge. An example of such an experiment is shown in Fig. 2A. Note that, in this example, TCEP treatment decreased the second and third population spike amplitudes possibly because of a desynchronization of the epileptiform discharge [5,17]. Nevertheless, pooled data (Fig. 2B; $n = 6$) show that TCEP did not significantly modify the amplitude of the first four population spikes, 101 ± 7%, 82 ± 33%, 69 ± 20% and 101 ± 38% of control, respectively ($P > 0.05$ in all cases). Subsequent treatment with DTNB did not modify the amplitude of the first population spike (98 ± 9% of control) whereas the second (25 ± 9%), third (9 ± 5%) and fourth (14 ± 12%) population spikes were considerably decreased. Finally, following application of D-APV, the first population spike remained unchanged (85 ± 9% of control) whereas the remaining population spikes were further reduced, 3 ± 1% for the second and 0% for the third and fourth. These results suggest that, in lesioned slices, NMDA receptors are in a fully reduced configuration since TCEP had little effect on the epileptiform activity. Pre-treatment with TCEP did not prevent the DTNB-induced decrease in evoked epileptiform discharges.

Several transmitter-gated ion-channel receptors contain cysteine residues and different types of redox modulation have been described (see Ref. [8]). In non-lesioned CA1 area of the hippocampus, DTNB does not affect AMPA, presynaptic metabotropic glutamate receptor, $GABA_A$ and $GABA_B$ receptor-mediated responses [10]. In contrast, NMDA receptors activity can be modulated up to 50% by redox compounds [8,9]. Since we have found that an oxidizing agent (DTNB) decreases the expression of excitatory events, while a reducing agent (TCEP) reinstated them, it is unlikely that such effects were mediated by a modification of the GABAergic (inhibitory) drive. Moreover, inhibition does not seem to be functional in this tissue [18]. We consider the NMDA receptors as the most likely target of the redox compounds used in this study to explain our results.

In the CA1 area of KA lesioned rat hippocampus, pyramidal cells show a graded bursting activity which is directly controlled by the activation of NMDA receptors [2,18]. We have used extracellular recordings of population spikes because they represent the best measure of the number and the degree of synchrony of bursting neurons in a localized area of the neuronal network. The first population spike and a small part of the second one result from the activation of AMPA receptors. All the other population spikes depend upon the activation of NMDA receptors. This is clearly shown by the complete block of the epileptiform discharge by D-APV. In our preparation, DTNB did not modify the first population spike. This is in keeping with the fact that AMPA receptor-mediated responses are not affected by redox reagents [1,9]. However, the second and subsequent population spikes were considerably decreased. This reduction was not due to a non-specific effect of the drug since TCEP reinstated the epileptiform activity to pre-DTNB levels. Conversely, when naive slices were treated with TCEP, epileptiform activity was not modified apart from a desynchronization of the epileptiform discharge which may be due to Ca^{2+} spikes [5,17] or a desensitization of NMDA receptors via a large Ca^{2+} influx [13]. These results suggest that native NMDA receptors are in a fully reduced configuration in this type of epileptic tissue.

In this study, we have obtained indirect evidence of the effect of DTNB. We have assumed that DTNB is acting only on CA1 pyramidal cell NMDA receptors. However, redox reagents could also affect the firing thresholds of the cells, a property described following electrical conditioning stimuli [5]. However, it must be noted that the first population spike of the epileptiform discharge remained unaffected by DTNB treatment, suggesting that the firing threshold was not modified, at least following activation of AMPA receptors. The direct effects of DTNB on NMDA receptor-mediated responses need to be investigated. In contrast, in non-lesioned tissue, DTNB always increased the population spike amplitude as previously described after D-APV treatment [6]. Since inhibitory interneurons receive a large excitatory input mediated by NMDA receptors [11], it is likely that a modification of the redox sites of NMDA receptors on interneurons will change the inhibitory drive in normal tissue.

In conclusion, we propose that the redox sites of NMDA receptors are good candidates upon which to direct treatments intended to reduce epileptiform activity. Redox reagents may not have deleterious effects since (1) they exist endogenously, and (2) redox reagent treatment

has been successfully used to reduce infarcts in rats (see Ref. [8]). It remains to be determined in this animal model of TLE, whether or not the pharmacological oxidation of NMDA receptors prevents synaptic plasticity (currently under study). This issue is fundamentally important because of its relationship to memory and learning processes. Finally, if redox agents are to have a therapeutic use, our results must be confirmed in epileptic tissue excised from human patients.

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