NMDA receptor redox sites: are they targets for selective neuronal protection?

Henri Gozlan and Yehezkel Ben-Ari

NMDA receptors play a central role in neuronal plasticity and in several pathological situations. Transient activation of this receptor triggers long-term potentiation, whereas sustained activation leads to cell death. Evidence for control of this activity by a redox site in cell cultures, brain tissues and in recombinant NMDA receptors are discussed by **Henri Gozlan and Yehezkel Ben-Ari**. The characteristics of this modulation and the consequences of redox state modifications on NMDA-mediated events are examined *in vitro* under physiological and pathological conditions. Since metabolic disorders enhance NMDA receptor function, the redox site could constitute a new target for selectively preventing *in vivo* the deleterious consequences of overactivation without blocking neuronal plasticity mediated by NMDA receptors.

Among the transmitter-gated ion channel family, the NMDA receptor occupies a special place, mainly due to its high permeability to Ca^{2+} (Ref. 1). This excitatory amino acid receptor controls several important physiological processes initiated by changes in $[Ca^{2+}]_i$, including long-term potentiation (LTP) and depression. However, excessive $[Ca^{2+}]_i$ resulting from overactivation of NMDA receptors, such as in ischaemic insults and epileptic disorders, triggers pathological processes that lead to neuronal death. Therefore, the regulation of NMDA receptor activity is important in the CNS, and this is achieved through several intra- and extracellular sites. Thus, glycine, histamine, steroids, polyamines, H^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, kinases, phosphatases and also redox drugs, modulate NMDA receptor-mediated responses[1].

Redox modulation of NMDA receptors in cell culture

Redox modulation of receptor responses is not a specific property of NMDA receptors, and several other transmitter-gated ion channels are also sensitive to redox conditions. However, NMDA receptors display some redox characteristics that are clearly distinct from those of other receptors (see Box 1). Most of the knowledge on NMDA receptor redox sites comes from work performed on cultures of cortical cells[2–5], cerebellar granules cells[6,7], and forebrain[8,9]. In these cells, disulphide-reducing agents, such as dithiothreitol (DTT), enhance, and thiol-oxidizing agents, such as 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), inhibit (but not completely) NMDA-evoked currents. This redox cycle can be repeated, indicating that the same sites are affected by oxidation and reduction.

On the basis of the nonpermeant properties of DTT and DTNB[2,4,10], these sites have been suggested to be located in the extracellular domain of the NMDA receptor. The fact that both reducing and oxidizing agents modify native NMDA receptor-evoked currents suggests that NMDA receptors are in equilibrium between a fully oxidized (disulphide) and a fully reduced (thiol) form. This equilibrium is dependent upon experimental conditions and also the presence of endogenous redox compounds[2] (see Box 2). Interestingly, potentiation of NMDA-mediated responses by DTT is not modified by *N*-ethylmaleimide (NEM), which irreversibly alkylates (mainly) cysteine residues[4]. Thus, conformational changes consecutive to disulphide bridge reduction, and not the availability of free sulphydryl groups, are responsible for the increase of the activity of NMDA receptors in cortical cells in culture. This observation probably rules out a role of these free sulphydryl groups in chelating metal ions in this preparation. DTT increases NMDA-mediated responses in an apparent concentration-dependent manner. The amplitude of NMDA-evoked currents is not significantly affected by 0.3 mM DTT, but is increased more than twofold by 4 mM DTT. Above this concentration a different response occurs, which could be another redox process but has not yet been fully characterized[4,8]. The onset of the reduction reaction (half-life of reaction approximately three minutes) is unaffected by the concentration of the reducing agent and proceeds slower than receptor activation[4], in agreement with a conformational change occurring as a result of a chemical reaction at a disulphide bridge. Potentiated NMDA-evoked currents are partially reversed by perfusing with buffer, but can be completely and quickly reversed by the addition of DTNB. This indicates that at least two redox states are involved in the modulation of NMDA receptor responses. This observation has been confirmed using isolated recombinant NMDA receptors[11–13].

Treatment with DTT appears to have limited consequences on NMDA receptor characteristics. On its own, DTT has no affinity for NMDA binding sites labelled with [^{3}H]tenocyclidine[5], and has no effect on glycine and polyamine modulation[8] of agonist responses. Its weak effect on agonist characteristics are disputed[4,8,14], but the possible increase in agonist affinities by DTT is not solely explained by its potentiation of NMDA-mediated responses, since this redox potentiation persists in the presence of saturating concentrations of agonists[4,8,13]. Indeed, single-channel activity indicates that DTT increases the frequency of single-channel opening[4,15] without affecting the mean open time or single-channel conductance[4]. The comparison between NMDA receptors oxidized using DTNB or alkylated using NEM shows no change in glycine and H^+ potentiation, whereas the

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Box 1. Redox modulation of transmitter-gated ion channels

The structure and the functions of several membrane-bound receptors containing Cys residues are highly dependent on conditions affecting thiol-disulphide exchanges. For instance, transmitter-gated ion-channel receptors contain well conserved disulphide bridges and different types of redox modulations have been observed. Thus, dithiothreitol (DTT) decreases nicotinic acetylcholine receptor[1] and glycine receptor[2] responses, but has only a modest effect on GABA-evoked currents[2], and does not modify 5-HT_3, AMPA or kainate receptor characteristics[3,4]. The only receptor to be clearly potentiated by DTT is the NMDA receptor[4].

The role of conserved cysteine residues is also different in NMDA receptors compared to the other transmitter-gated ion-channel receptors. In the nicotinic acetylcholine receptor, four Cys residues are thought to form two distinct extracellular disulphide bridges. They are both located in the *N*-terminal domain of the receptor subunits. The first disulphide bridge occurs in all subunits between two consecutive Cys residues (Cys192–Cys193) in the α subunit, and is involved in the recognition of the agonist[1,5]. Similar adjacent Cys residues were also found in NR1 (Cys454–Cys455) (Fig. 2 main text) and NR2 subunits, but their replacement by other residues has no effect on both agonist affinity and DTT potentiation[6–8]. The second pair of Cys residues in the nicotinic acetylcholine receptor (Cys128 and Cys142, in the α subunit) are separated by 13 amino acids, eight of these amino acids are conserved in $GABA_A$, glycine and 5-HT_3 receptors[9]. In NR1 subunit, a disulphide bridge between Cys420 and Cys436 has been shown to be important for glycine modulation[6]; although of comparable size (17 residues), the sequence between Cys420 and Cys436 is, however, completely different from that of other transmitter-gated ion channels. Finally, excitatory amino acid receptors contain another set of cysteine residues (Cys744 and Cys798; in the NR1 subunit) that are well conserved within all NR2 subunits, AMPA and kainate receptor sequences. According to the proposed topology of these receptors[10], the Cys residues are localized in the extracellular domain. However, a functional disulphide bridge involving these residues was only found in NMDA receptors composed of NR1 and either NR2B, NR2C or NR2D subunits and not in ones with NR1 and NR2A subunits or in the other excitatory amino acid receptors[8]. Interestingly, redox modulation and disulphide bond location also indicate that NMDA receptors belong to a receptor family different from that defined by the nicotinic acetylcholine receptor.

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Mg^{2+}-mediated block and Zn^{2+}-mediated inhibition are slightly decreased for alkylated receptors[4]. However, the link between DTT-mediated potentiation and removal of Mg^{2+}-mediated block is still unclear.

Redox modulation of NMDA receptors in brain slices

Redox modulation of NMDA receptors has also been observed in several tissue preparations including intact chick retina[2], rat hippocampal[16–18], cortical and striatal[19] slices, and post-mortem human cortex[20]. In hippocampal slices the redox modulation of pharmacologically isolated NMDA receptors has been studied using intracellular, extracellular, and patch-clamp recordings[16,21] (Fig. 1). In the CA1 region of the hippocampus, NMDA receptors are less sensitive to reduction than to oxidation, since a concentration of 200 μM of any reducing agent produced only a modest potentiation[16], whereas 50 μM DTNB produces the maximal inhibition (50%) of NMDA receptor-mediated responses[16]. Higher concentrations of DTNB do not increase the level of oxidation but decrease the time required to reach this maximal level[16]. This long-lasting inhibition can be reversed by tris(2-carboxyethyl)phosphine (TCEP)[16,21], and by DTT, two chemically different and potent disulphide-reducing agents, but not by extensive washing of the preparation. The effects of redox agents are also observed in modulation of NMDA receptor-mediated responses on isolated hippocampal outside-out patches, showing that intracellular events are not involved in this process.

Interestingly, in both neuronal cell cultures and in brain slices, the fact that fully oxidized NMDA receptors are still functional indicates that redox modulation occurs to control the overactivity of NMDA receptors induced by a reductive process and not to affect their basal responses.

Redox modulation of recombinant NMDA receptors

The modulation of homomeric NR1 subunits in NMDA receptors expressed in *Xenopus* oocytes by DTT or DTNB is either absent[13] or of small amplitude[12], suggesting that the formation of a functional disulphide bond requires a NR2 subunit or another protein. Indeed, responses of recombinant heteromeric NMDA receptors containing NR1 and NR2 subunits expressed in human embryonic kidney (HEK293) cells[11] and *Xenopus* oocytes[12,13,22] are potentiated by DTT and inhibited by DTNB. These processes are highly dependent on the type of NR2 subunit[11,13]. Indeed, based on pharmacological and kinetic studies, three different sets of redox sites with different

Box 2. Endogenous redox regulators of NMDA receptors

In neuronal preparations, NMDA receptors exist as an equilibrium between at least two redox states: one fully oxidized with a moderate activity and one fully reduced, having a higher efficacy (Fig. 3 main text). The existence and variations in the proportions of these two states in different preparations suggests the presence of endogenous redox compounds that maintain the redox equilibrium. Several candidates have been suggested to play such a role, because of their *in vitro* documented effects on NMDA receptors:

Thiol and disulphide reagents

Several thiol derivatives, cysteine[1], homocysteine[2] and dihydrolipoic acid[3], increase NMDA receptor-mediated responses. Oxidizing compounds such as lipoic acid[3], a cyclic disulphide, and methoxatin, a quinone derivative that can react with free sulphydryl groups[4] and metal ions[1,5,6], inhibit NMDA receptor-mediated responses. The effects of glutathione (GSH) and its oxidized form (GSSG) on NMDA receptor function are less clear, probably because these drugs are frequently contaminated with glutamate and cysteine, and also because they display some agonist properties at NMDA receptors[7]. Cysteine and homocysteine are known to be released during depolarization[8] and could contribute to and maintain the NMDA receptor in a reduced state. Interestingly, during metabolic stress, and particularly during anoxic episodes, oxygen deprivation displaces the redox state of the cell toward a more reduced state and cysteine concentration increases[9,10]. The enhancement of glutamate release could also modify cysteine and GSH metabolism[11,12]. Therefore, if the concentration of these compounds is high enough in the extracellular space, they could contribute to an overstimulation of NMDA receptors *in vivo*, leading to deleterious effects.

Ascorbic acid

Ascorbic acid, a very common endogenous reducing agent, does not potentiate, but inhibits, NMDA-evoked currents[13]. Dehydroascorbic was unable to inhibit NMDA-evoked currents, ruling out an effect through oxidation of ascorbic acid[13]. Furthermore, ascorbic acid also inhibits glutamate-induced toxicity in neuronal cells[14] and NMDA receptor-dependent release of acetylcholine[15]. Another reducing agent, hydroquinone, behaves similar to ascorbic acid, and both inhibitions appear not to be mediated through the H^+ regulatory site of the NMDA receptor[13], but are proposed to result from a conformational change produced by alteration of the electric charge of the NMDA receptor[13].

Free radicals

Free radicals are also oxidizing compounds known to react with thiol groups and indeed, oxygen free radicals generated by the activation of xanthine oxidase inhibit NMDA receptor-mediated responses[16]. The site of action of these free radicals has not been determined, but

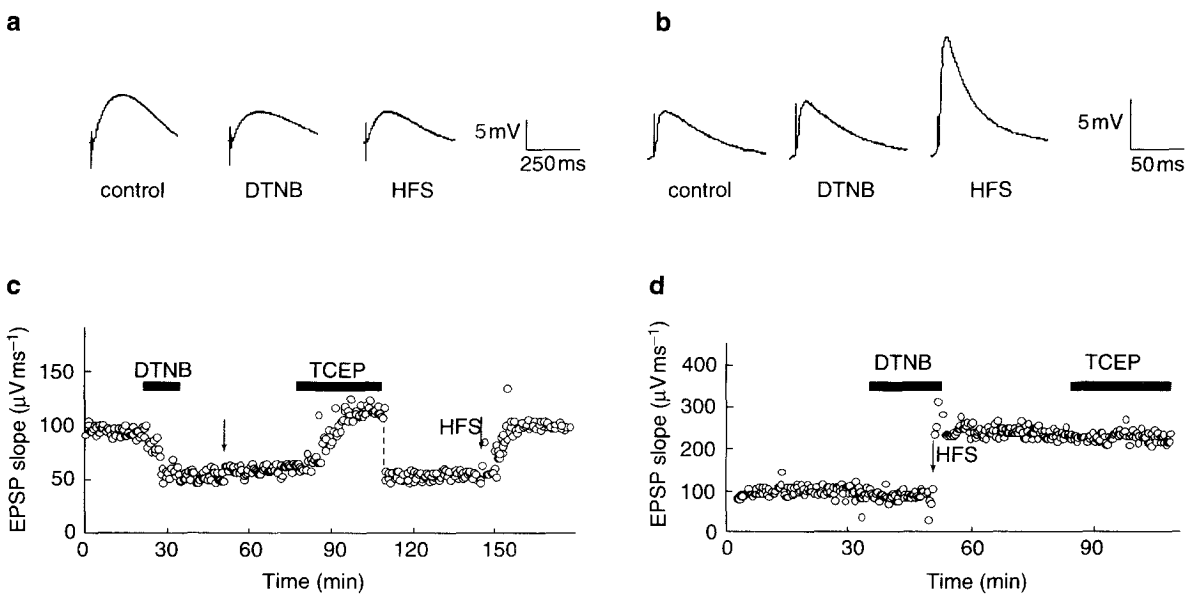

Fig. 1. Effect of redox agents on the induction of long-term potentiation (LTP) of NMDA and AMPA receptors in the CA1 region of the rat hippocampus. **a, b:** Intracellular recordings: experiments were performed at −67 mV in the presence of $MgCl_2$ (1.3 mM) and $CaCl_2$ (2 mM). a: 5,5′-dithiobis-2-nitrobenzoic acid (DTNB; 200 μM, 10 min) inhibits NMDA receptor-mediated excitatory postsynaptic currents (EPSC). In the presence of DTNB or 30 min after DTNB application, no LTP of NMDA receptors could be generated by high frequency stimulation (HFS; 4 × 100 Hz, 1 s). b: AMPA receptor-mediated EPSC are not significantly affected by DTNB (200 μM, 10 min), and in the presence of DTNB a LTP of AMPA receptors was generated by HFS (4 × 100 Hz, 1 s). **c, d:** Extracellular recordings: c: DTNB (200 μM) inhibits NMDA receptor field excitatory postsynaptic potentials (EPSP). HFS (3 × 100 Hz, 1 s) 15 min after DTNB (arrow) did not induce LTP of NMDA receptors. Tris (2-carboxyethyl)phosphine (TCEP; 200 μM, 30 min) increases NMDA receptor field EPSP. The level of stimulation was then adjusted (dotted line) to that obtained in the presence of DTNB, and HFS (3 × 100 Hz, 1 s) now induces LTP of NMDA receptors. d: DTNB (200 μM) has no effect on the slope of AMPA field EPSP and did not prevent the induction of LTP of AMPA receptors generated by HFS (3 × 100 Hz, 1 s) (arrow). Addition of TCEP (200 μM, 20 min) has no effect on AMPA receptor field EPSP.

particular attention has focused on NO, which also decreases NMDA receptor activity[17]. The mechanism of this effect was suggested to involve a direct action on free sulphydryl groups of the NMDA receptor redox site[17]. However, even when these sulphydryl groups were alkylated by *N*-ethylmaleimide (NEM), NO still demonstrates inhibiting properties on NMDA receptors[6,18,19]. This indicates that either NO acts on a different target, or that a second redox site, unchanged by NEM treatment, could be the locus of NO inhibition (or both). Recently, a metal ion, associated with NMDA receptors has been proposed to play a role in the effects of NO (Ref. 6).

Thus, several classes of endogenous redox compounds can modulate NMDA receptor-mediated responses, apparently by different mechanisms. Other yet unidentified redox compounds, including enzymes and proteins[20,21], could also affect redox states of NMDA receptors. Depending on the concentration and the turnover of these endogenous compounds, modifications of redox potentials during ischaemic episodes may lead, as observed *in vitro* experiments, to a long-lasting overactivation of NMDA receptors through redox sites, *in vivo*.

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accessibility to DTT and thermodynamic properties (Table 1) have been described in HEK293 cells[11], and largely confirmed in *Xenopus* oocytes[12,13]. DTT displays a 'classical' potentiation of NMDA receptors composed of a NR1 subunit and either a NR2B, NR2C or NR2D subunit, but two types of redox effects are found on NMDA receptors composed of NR1 and NR2A subunits[11]. Furthermore, DTT decreases desensitization of NMDA-evoked currents in the NMDA receptors (NR1 and NR2A subunits) and decreases the timecourse of the deactivation processes of this type of receptor, but increases that of a NR1 subunit and either a NR2B, NR2C, or NR2D subunit composed NMDA receptors. This indicates that in the presence of reducing agents, both the amplitude and the duration of the response are modified. This observation could have important functional consequences.

The Cys residues involved in the redox modulation of recombinant NMDA receptors have been identified for NR1, NR2B, NR2C and NR2D subunits[13]. Surprisingly, although DTT modulation requires a NR2 subunit, the two Cys residues involved in this effect (Cys744 and Cys798), are found in the NR1 and not in the NR2 subunit. According to the proposed topology of NMDA receptors[23], these residues have an extracellular location (Fig. 2). In contrast, DTT modulation of NMDA (NR1 and NR2A subunits) receptors is only slightly affected by the mutation of these residues and not modified by the mutation of other Cys residues in the NR1 or in the NR2A subunit. This confirms that several processes, involving more than one pair of Cys residues, are responsible for the redox properties of NMDA receptors in neuronal preparations. One of these is probably a thiol-disulphide exchange, and seems to be functional in NMDA (NR1 plus NR2B, NR2C or NR2D subunits) receptors. Potent reducing agents, such as dithiols and phosphine derivatives

Table 1. Characteristics of redox sites for recombinant NMDA receptors

Characteristic	NMDA receptor redox site		
	1	2	3
NR2 subunit	2A	2A	2B, 2C, 2D
Concentration of DTT (mM)	0.3	3	3
Potentiation by DTT (%)	210	220	180–200
Potentiation by monothiols	yes	yes	no
Onset of potentiation (min)	<1	30	30
DTT effects reversed by washing	yes	no	no
DTT effects reversed by DTNB	yes	yes	yes
Alkylation by NEM	no	no	yes
Inhibition by NEM	n.d.	yes	no
Desensitization after DTT	reduced	n.d.	n.d.
Deactivation after DTT	increased	unchanged	reduced
Cys residues in NR1[a]	n.d.	Cys744, Cys798	Cys744, Cys798

[a]Data from Ref. 13; data adapted from Ref. 11.DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; DTT, dithiothreitol; NEM, *N*-ethylmaleimide.

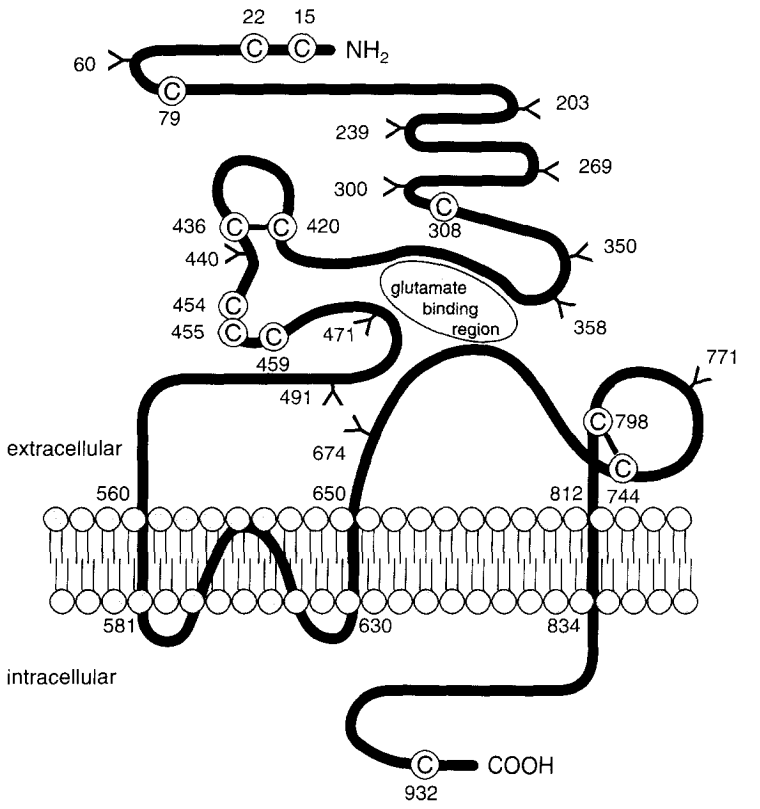

Fig. 2. Diagram of the NR1 subunit of the NMDA receptor. The NR1 subunit has three transmembrane (TM) domains and a channel-lining re-entrant membrane loop according to the presumed topology of excitatory amino acid receptors. Putative glycosylated Asn residues are numbered. Cys residues (C) that are conserved in NR1 and all NR2 subunits are coloured yellow. Two disulphide bridges are represented for the glycine agonist binding site (Cys420 and Cys436) and for redox site 3 (Cys744 and Cys798). The glutamate binding site was represented by analogy to the glutamate binding site of bacterial amino acid-binding proteins.

are required to cleave such disulphide bonds. This process probably also occurs in NMDA (NR1 and NR2A subunits) receptors but is masked by the participation of other Cys residues to another redox mechanism. Among several possibilities, the chelation of a metal ion by several Cys residues may be involved in the reversible potentiation of NR1 and NR2A subunit NMDA receptor by DTT (redox site 1). Indeed, thiol compounds are more potent in chelating metal ions than in disrupting disulphide bridges[24], and low concentrations of DTT or monothiol reagents can competitively inhibit the chelation of a metal ion by NMDA receptors.

Redox modulation of NMDA receptor function

$[Ca^{2+}]_i$ concentrations

At the cellular level, the activation of NMDA receptors rapidly increases $[Ca^{2+}]_i$ in neuronal cultures and this effect is enhanced in a concentration-dependent manner by DTT (Refs 6, 8, 9, 24, 25). There is also a good correlation between concentrations of DTT required to potentiate NMDA-evoked currents and to enhance $[Ca^{2+}]_i$ (Ref. 7) or $^{45}Ca^{2+}$ uptake in rat cerebellar granule cells[24]. This effect is mediated by NMDA receptors as competitive and noncompetitive NMDA receptor antagonists completely suppress the effect of DTT (Refs 7, 26). Interestingly, DTNB displays only a limited inhibition of Ca^{2+} influx[6,7], indicating again that the activity of the NMDA receptor cannot be completely suppressed by oxidation. The consequences of the reduction of the NMDA receptor on $[Ca^{2+}]_i$ are also mimicked by other thiol-containing compounds such as mercaptoethanol and glutathione[6]. The augmentation of $[Ca^{2+}]_i$-induced fluorescence[8] observed when the concentration of DTT is increased tenfold could occur through a second NMDA receptor redox but has not been investigated. These findings demonstrate that potentiation of NMDA-evoked currents through redox sites correlates with the influx of Ca^{2+} through the NMDA receptor–channel complex.

Neuronal toxicity

In agreement with the above conclusions are the effects of DTT and reducing agents on glutamate- (Refs 24, 25) or NMDA-induced toxicity[25,27] mediated through NMDA receptors. Indeed, potentiation of NMDA receptor-mediated responses by reducing agents is associated with an increase in neuronal death in different preparations[24,25,27]. This process is prevented by DTNB (Ref. 27), chelating agents[24], and competitive[25,27] and noncompetitive[24] NMDA receptor antagonists.

Neurotransmitter release

NMDA modifies the release of several neurotransmitters, and this property is also subject to redox modulation. Although the mechanisms of these effects are not completely elucidated, the addition of DTT to cortical, hippocampal and striatal slices results in an increase in the NMDA-evoked release of noradrenaline and dopamine, effects that are associated with an enhancement of the activity of the NMDA receptor through its redox modulatory sites[18,19]. Interestingly, DTT potentiation of NMDA-evoked neurotransmitter release is more marked in the striatum than in the cortex or the hippocampus[19], confirming that redox modulation is dependent on either the environment of the NMDA receptor or on its subunit composition (or both). Finally, DTNB does not completely suppress the NMDA-evoked release of neurotransmitters[19].

Synaptic plasticity

Activation of NMDA receptors is an essential triggering factor in the induction of LTP (Ref. 28), a form of synaptic plasticity generated by a high-frequency stimulation of synapses into which glutamate is released. In physiological conditions, this process transiently activates NMDA receptors, thus increasing $[Ca^{2+}]_i$, which activates a cascade of events leading to a persistent potentiation of the synaptic efficacy of AMPA receptors. When the Mg^{2+} block is removed or reduced, or when AMPA receptors are blocked[29,30] (or both), another form of LTP takes place that still requires the activation of NMDA receptors for its induction but it is the synaptic efficacy of NMDA receptors that is enhanced. A similar form of LTP occurs

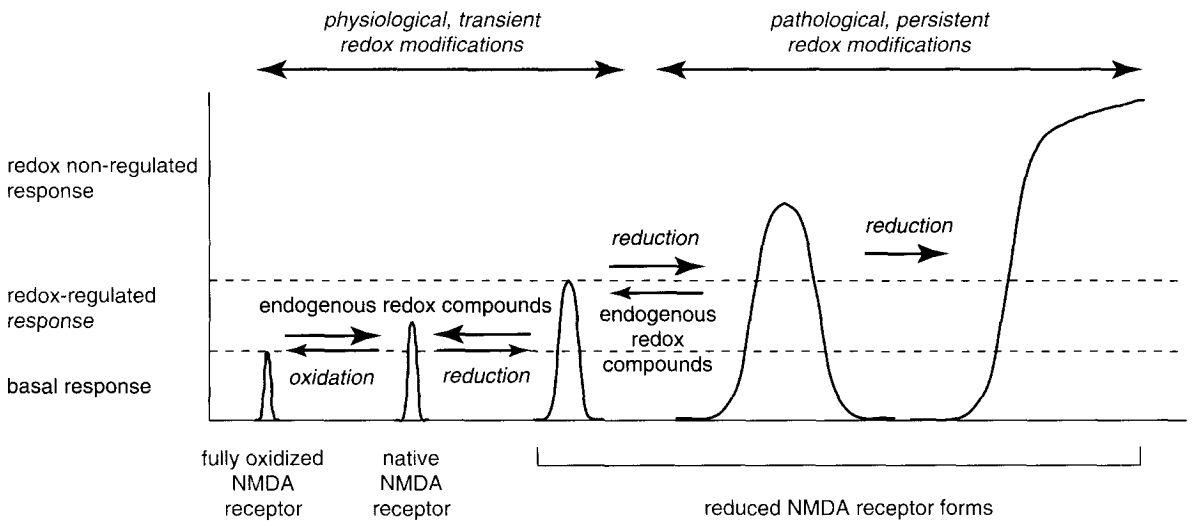

Fig. 3. NMDA receptor activity mediated by redox sites in physiological and pathological conditions. Depending on subunit composition and redox environment, the response of native NMDA receptor lies between a fully oxidized and a reduced form. The presence of endogenous redox compounds allows only transient modifications of NMDA receptor-mediated responses and this is within a limited redox regulated range. Redox site 1 might be involved in this control. A moderate reduction, slightly increases the duration and the amplitude of the NMDA receptor-mediated response, but endogenous redox compounds rapidly restore the native NMDA receptor signal. In several situations, the concentration of reducing agent is highly increased and could irreversibly affect a second redox site (redox site 2 or 3). This reducing environment overcomes the buffering capacity of the endogenous redox compounds and the duration and the amplitude of NMDA receptor-mediated response are now increased beyond the endogenous redox compound-regulated domain. Summation of these responses would induce the long-lasting response of NMDA receptors.

following a brief anoxic aglycaemic episode, mimicking *in vitro* the effect of an ischaemic event[31,32]. In contrast to tetanic LTP where NMDA receptor efficacy is enhanced, this LTP also occurs in the presence of physiological concentrations of Mg^{2+} (Refs 31, 32).

Since activation of NMDA receptors is instrumental for LTP in the CA1 region of the hippocampus, the consequence of redox manipulation of NMDA receptors on these different forms of LTP has been analysed in hippocampal slices[16,21,33] using intracellular, extracellular, and patch-clamp recordings with similar results (Fig. 1). Thus, DTNB and TCEP do not modify AMPA receptor-mediated responses[21,33]. In contrast, DTNB produced a 50% inhbition of the responses mediated by NMDA receptors[16]. This effect is irreversible, indicating that under these *in vitro* conditions, endogenous reducing agents are in a limited concentration or display only weak reducing properties (see Box 2). Although NMDA receptors activation is a pre-requisite for LTP in the CA1 region of the hippocampus, their oxidation by DTNB, and subsequent the 50% inhibition of their activity, neither prevents the induction nor the expression of LTP with potentiation of AMPA receptor efficacy (Ref. 33). NMDA receptor redox sites are therefore not involved in this physiological event (Fig. 1b, d), thought to be involved in cognitive processes[34]. Likewise, long-term depression of AMPA receptors, another form of synaptic plasticity requiring also activation of NMDA receptors, is not affected by oxidation[35].

In contrast, the induction and the expression of LTP with increased synaptic plasticity of NMDA receptors[33] are prevented by DTNB and reversed by disulphide-reducing agents (Fig. 1a, c). This process is related to a redox modification and not to a partial inhibition of NMDA receptors by DTNB because: (1) increasing the level of stimulation during the tetanic stimulation of oxidized slices does not restore the induction of LTP; and (2) the same amount of blockade (50%) by a noncompetitive NMDA receptor antagonist dizocilpine does not prevent the induction and expression of LTP (Ref. 33).

Interestingly, DTNB also prevented the induction of LTP following an anoxic episode, and this form of plasticity can be restored by reducing agents, suggesting that NMDA receptor redox sites have been affected during anoxic episodes[16].

These series of experiments indicate that long-lasting changes in NMDA receptor-mediated responses cannot take place when the receptor is maintained in a fully oxidized state, and suggest that NMDA receptors must be shifted toward a more reduced state in order for persistent alterations in NMDA receptor-mediated events to occur[32].

Ischaemic and epileptic disorders

In hippocampal slice preparations, DTT markedly increases neuronal excitability[36] and evidence exists in favour of hyperactivation of NMDA receptors in epileptic[37,38] and ischaemic disorders[31,39–41]. In the latter, hyperactivation is at least partly mediated by NMDA receptor redox sites, as suggested by: (1) the prevention by DTNB of LTP induced by anoxic episodes; and (2) the correlation between regional vulnerability of brain tissue to hypoxic conditions[42] and the potency of redox modulation of NMDA receptors in these areas[19]. Indeed,

during ischaemic episodes, the increase in glutamate induces neuronal and glial modifications of the release and metabolism of several endogenous thiol compounds (including glutathione and cysteine)[43–45], which can shift the redox state of the cell towards a more reduced potential.

Thus, together with a loss of Mg^{2+} block[46], these processes may be predicted to induce a long-lasting activation of NMDA receptors *in vivo*. However, most of the available data is from *in vitro* experiments, hence the functional relevance of redox modulation of NMDA receptors must await direct evaluation with *in vivo* models of global and focal ischaemia and epilepsy.

Preliminary observations showing that DTNB blocks, and TCEP enhances, the spontaneous hippocampal paroxysmal activity in a rat model of temporal lobe epilepsy produced by i.c.v. injections of kainic acid are encouraging. Furthermore, the oxidizing compound methoxatin, which has been shown *in vitro* to inhibit NMDA receptor-mediated responses through redox sites[47] (see Box 2), reduces the infarct in a rat model of hypoxic ischaemic injury[48].

Perspectives

Redox modulation of NMDA receptor activity is a complex process that involves several extracellular sites regulated, at present, by unknown endogenous compounds. Interestingly, fully oxidized NMDA receptors are still functional, indicating that redox modulation does not work as an all-or-nothing switch, but acts rather as a buffer of NMDA receptor hyperactivity. A working hypothesis is that under physiological conditions, this system (which includes NMDA receptor redox sites and endogenous redox compounds) rapidly buffers small changes in redox potential that occur in relation to electrical activity, maintaining the activity of NMDA receptors within a controlled range (Fig. 3). However, the buffering capacity of this system cannot rapidly overcome large variations of redox potential that occur in pathological conditions, and particularly when they are associated with exacerbated electrical activity and with modifications of other regulatory process, such as Mg^{2+} block. High concentrations of reduced compounds (mainly monothiols) would irreversibly affect another redox site (mainly redox site 2) and considerably increase the amplitude and the duration of NMDA receptor-mediated signals. The consequence of this effect is a long-lasting enhancement of NMDA receptor-mediated responses (Fig. 3).

If this model is confirmed *in vivo*, it may open new perspectives for the treatment of the various pathological consequences of overactivity of NMDA receptors. In fact, regulation of redox sites and other modulatory sites of NMDA receptors may be more beneficial than direct blockade of the receptor–channel complex, because of the side-effects of competitive and noncompetitive NMDA receptor antagonists[49]. This is particularly warranted if oxidizing agents could block the excessive potentially toxic action of NMDA receptors without preventing LTP and other memory processes mediated by this receptor.

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