Molecular Psychiatry (2002) 7, 32–43

www.nature.com/mp

ORIGINAL RESEARCH ARTICLE

Receptor mechanisms and circuitry underlying NMDA antagonist neurotoxicity

NB Farber[1], SH Kim[2], K Dikranian[1], XP Jiang[1] and C Heinkel[1]

[1]*Department of Psychiatry, Washington University, St Louis, MO 63110, USA;* [2]*Department of Psychiatry, Guro Hospital, College of Medicine, Korea University, Seoul, 152–703, Korea*

NMDA glutamate receptor antagonists are used in clinical anesthesia, and are being developed as therapeutic agents for preventing neurodegeneration in stroke, epilepsy, and brain trauma. However, the ability of these agents to produce neurotoxicity in adult rats and psychosis in adult humans compromises their clinical usefulness. In addition, an NMDA receptor hypofunction (NRHypo) state might play a role in neurodegenerative and psychotic disorders, like Alzheimer's disease and schizophrenia. Thus, understanding the mechanism underlying NRHypo-induced neurotoxicity and psychosis could have significant clinically relevant benefits. NRHypo neurotoxicity can be prevented by several classes of agents (eg antimuscarinics, non-NMDA glutamate antagonists, and α_2 adrenergic agonists) suggesting that the mechanism of neurotoxicity is complex. In the present study a series of experiments was undertaken to more definitively define the receptors and complex neural circuitry underlying NRHypo neurotoxicity. Injection of either the muscarinic antagonist scopolamine or the non-NMDA antagonist NBQX directly into the cortex prevented NRHypo neurotoxicity. Clonidine, an α_2 adrenergic agonist, protected against the neurotoxicity when injected into the basal forebrain. The combined injection of muscarinic and non-NMDA Glu agonists reproduced the neurotoxic reaction. Based on these and other results, we conclude that the mechanism is indirect, and involves a complex network disturbance, whereby blockade of NMDA receptors on inhibitory neurons in multiple subcortical brain regions, disinhibits glutamatergic and cholinergic projections to the cerebral cortex. Simultaneous excitotoxic stimulation of muscarinic (m_3) and glutamate (AMPA/kainate) receptors on cerebrocortical neurons appears to be the proximal mechanism by which the neurotoxic and psychotomimetic effects of NRHypo are mediated.
Molecular Psychiatry (2002) **7,** 32–43. DOI: 10.1038/sj/mp/4000912

Keywords: neurodegeneration; retrosplenial cortex; MK801; dissociative anesthetics; Alzheimer's disease; schizophrenia; psychosis; rodents; rats

Introduction

Excessive activation of NMDA glutamate receptors triggers neuronal degeneration in acute brain injury conditions, such as stroke, head trauma and epilepsy, and NMDA antagonists confer protection against such degeneration. However, NMDA antagonists have significant side effects. In adult humans they trigger psychosis,[1–10] and in adult rats they produce neurotoxicity.[11–21] Developing methods for controlling or preventing these adverse reactions is an important goal, as it may permit the therapeutic potential of these agents to be realized.

In addition to the above, there are other important reasons for studying these effects of NMDA antagonists. Because the psychotic reaction triggered by these agents resembles the psychotic manifestations of schizophrenia,[1,2] exploring the mechanisms by which these drugs disrupt neural circuitry may shed new light on the nature of the network disturbances operative in schizophrenia.[22,23] NMDA receptor hypofunction (NRHypo), the condition induced in the brain by NMDA antagonist drugs, is a condition that is endogenously present in the aging brain,[24–27] and is present to an exaggerated degree in the brains of patients with Alzheimer's disease (AD).[28] For this reason, and because of certain parallels between the pattern of neurodegeneration in AD and the pattern induced in rat brain by NMDA antagonists, it has been proposed that NRHypo may act in concert with amyloidogenic mechanisms to drive the neuropathological process in AD.[29–31] Finally, it is important to understand both the pathogenesis and ways of preventing NRHypo neurotoxicity, because certain drugs used in clinical anesthesia (ie ketamine and nitrous oxide (laughing gas)) are NMDA antagonists.

The pathomorphological changes induced by

Correspondence: NB Farber, MD, Department of Psychiatry, Washington University, Campus Box 8134, 660 S Euclid Ave, St Louis, MO 63110–1093, USA. E-mail: farbern@psychiatry.wustl.edu
Received 6 November 2000; revised 16 February 2001; accepted 23 February 2001

NRHypo have been shown to be either reversible or irreversible, depending on how long NMDA receptor blockade is maintained. Changes induced by a low dose of an NMDA antagonist are reversible, are regionally selective for neurons in the retrosplenial cortex (RSC[32]), and consist of vacuolar changes in mitochondria and endoplasmic reticulum.[11] A high dose, or more prolonged treatment, triggers irreversible neurodegeneration affecting neurons not only in RSC,[12–16] but also in several other cerebrocortical and limbic brain regions.[17–19,21]

After finding in early experiments, that two classes of drugs—$GABA_A$ agonists and muscarinic cholinergic antagonists—were effective in blocking the reversible neurotoxic reaction, we proposed (Figure 1) that the mechanism involves loss of GABAergic inhibitory control over excitatory cholinergic neurons that innervate RSC neurons, with excessive cholinergic stimulation of RSC neurons being the proximal mechanism of neuronal injury.[13] Subsequent findings have implicated several other transmitter receptor systems in the neurotoxic reaction,[33–35] suggesting that the underlying mechanism may be much more complex. In the present study, we have undertaken a series of additional experiments designed to determine more definitively the receptor mechanisms and complex neural circuitry that mediate NRHypo neurotoxicity.

Materials and methods

Methods

Adult female Sprague–Dawley rats were anesthetized with isoflurane to permit stereotaxic injection of neuroactive substances through a burr hole into the RSC (AP: –5.5, ML: +0.8, DV: –1.6[36]), anterior cingulate cortex (AP: +1.2, ML: +0.4, DV: –2.0), the isocortex lateral to RSC (AP: –5.6, ML: +4.0, DV: –2.0), vertical limb of the diagonal band of Broca (AP: +0.7, ML: +0.2, DV: –7.4), horizontal limb of the diagonal band of Broca (AP: –0.26, ML: +1.8, DV: –9.0), nucleus basalis (AP: –1.4, ML: +2.4, DV: –7.0), nucleus accumbens shell (AP: +1.0, ML: +0.8, DV: –7.4), or locus coerulus (AP: –9.8, ML: +1.3, DV: –7.2). Glass micropipettes pulled to an orifice diameter of approximately 25–50 μm were used for the microinjections. In experiments entailing injections of protective agents into cortex, the agent of interest was injected in a volume of 1.0 μl. For noncortical areas the injectate volume was 0.1 μl. Immediately after the intracranial injection, the scalp wound was closed, anesthesia was discontinued and MK801 was administered subcutaneously (sc) in a dose (0.5 mg kg^{-1}) sufficient to induce a robust vacuole reaction in RSC. When isoflurane anesthesia is terminated immediately prior to the time of MK801 administration, it does not interfere with the neurotoxic reaction[37] because isoflurane is rapidly cleared by the lungs. Four hours after the injection of MK801, the animals were deeply anesthetized with chloral hydrate and perfused with fixative (4% paraformaldehyde, 1.5% glutaraldehyde in phosphate buffer at pH 7.0) through the ascending aorta. The brains were cut into 1-mm thick transverse slabs which were post fixed in osmium tetroxide (1%), dehydrated in a series of ethanols, cleared in toluene and embedded flat in araldite.

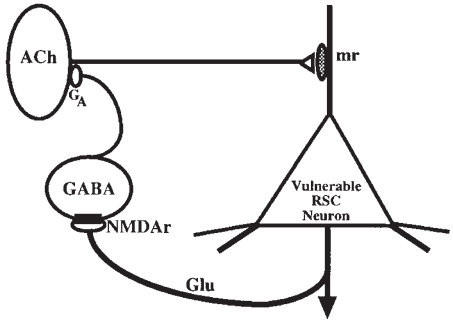

Figure 1 Circuitry originally proposed to mediate NRHypo neurotoxicity. Based on early evidence that NRHypo neurotoxicity could be blocked by $GABA_A$ agonists or muscarinic cholinergic antagonists, we proposed a simple wiring diagram to explain NMDA antagonist neurotoxicity. It was postulated that blockade of NMDA receptors on GABAergic interneurons inactivated these neurons, thereby disinhibiting release of ACh at a muscarinic receptor on the RSC neuron as the proximal mechanism of injury. ACh = acetylcholine; Glu = glutamate; GABA = γ-amino butyric acid; G_A = $GABA_A$ subtype of GABA receptor; mr = muscarinic cholinergic receptor; NMDAr = NMDA subtype of Glu receptor.

For light microscopic evaluation, histological sections were cut 1 μm thick with an MT 2C Sorvall Ultratome using 0.5 inch wide glass knives. These sections were stained with a mixture of methylene blue and Azure II for light microscopic evaluation. The brains were sectioned at several rostrocaudal levels to permit localization of the injection site and quantitative evaluation of the vacuole reaction in RSC. For the latter purpose, depending on the site of injection, vacuolated neurons were counted bilaterally at one of two different levels. For those animals that received the injection into the RSC, counts were obtained from the section closest to the injection site (Figure 2a). For injections outside of the RSC, counts of injured neurons from the RSC were obtained at the level where the neurotoxic reaction is known to be most severe (approximately 5.5 mm caudal to Bregma, a level which is easily located because it is where the corpus callosum ceases decussating across the midline; Figure 2g). The investigator quantifying the reaction was blind to the treatment conditions. Percent protection was calculated by dividing the mean of vacuolated neurons on the side ipsilateral to the injection site by the mean number on the contralateral side. The result was subtracted from 1 and multiplied by 100.

For electron microscopy, ultrathin sections were cut and suspended over a formvar coated slot grid (1 × 2 mm opening), stained with uranyl acetate and lead citrate and viewed in a JEOL 100 C transmission electron microscope.

In those experiments investigating the ability of certain agent(s) to mimic NMDA antagonist neurotoxicity when injected directly into RSC, the protocol was as described above except that systemic administration of

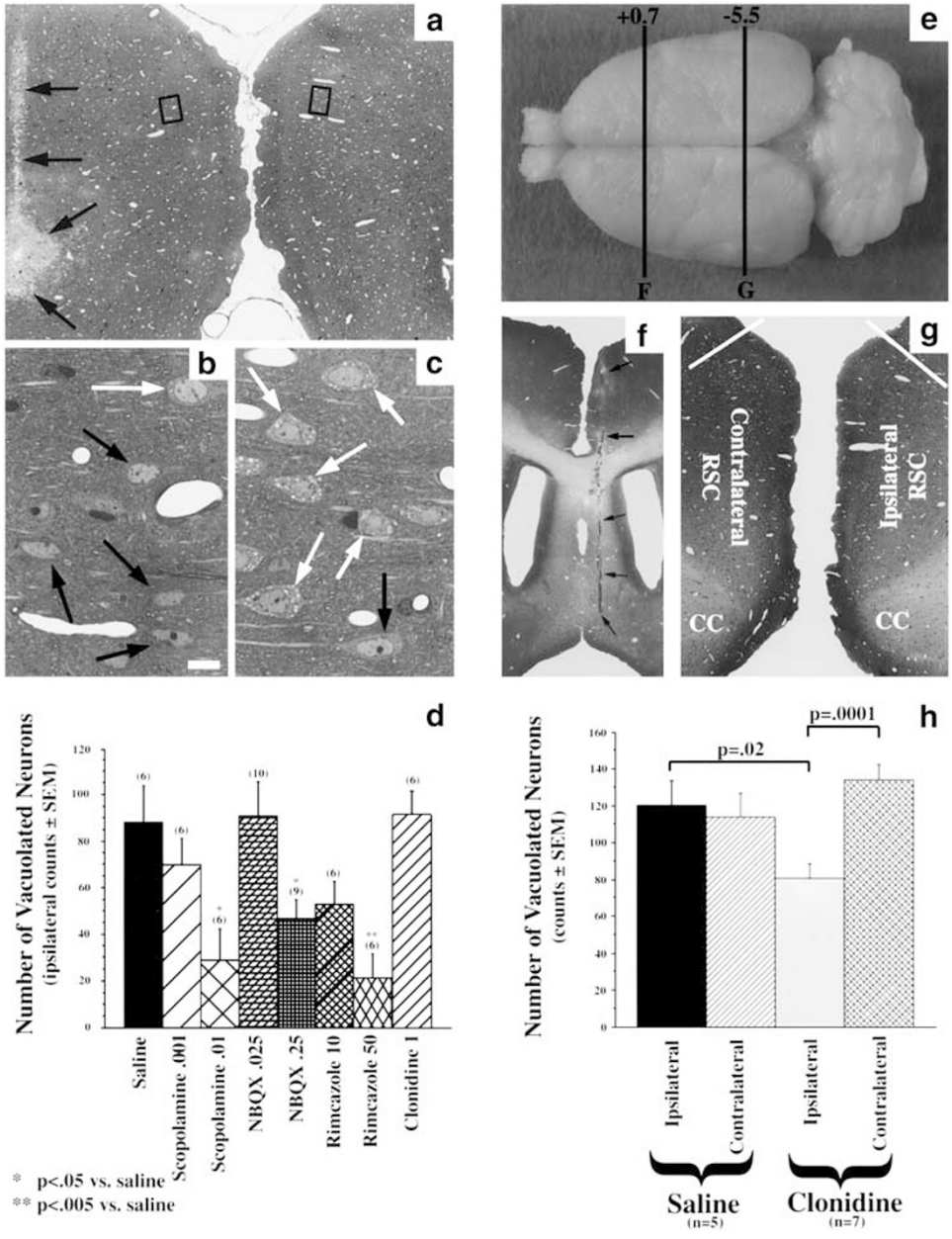

Figure 2 Blockade of NRHypo neurotoxicity by injection of protective agents into brain. (a) Low power magnification of the RSC of a rat treated with MK801 systemically plus scopolamine directly injected into the RSC. Arrows indicate the track of the needle that was used to inject scopolamine. Boxes indicate location of areas that are shown in panels (b) and (c). (b) Light micrographic scene from RSC ipsilateral to the injection site. The majority of pyramidal neurons do not have vacuoles and are normal in appearance (black arrows). One neuron remains affected (white arrow). White scale bar = 10 μm. (c) Light micrographic scene from RSC contralateral to the injection site. The majority of layer III–IV pyramidal neurons (white arrows) are injured and show the presence of vacuoles. One neuron is not affected (black arrow). Magnification is the same as that in panel (b). (d) Bar graph illustrating the amount of protection against NRHypo neurotoxicity provided ipsilaterally by the injection of different agents directly into the RSC. Compared to saline, scopolamine provided protection against NRHypo neurotoxicity (treatment condition × side: $F[2,15] = 11.07$, $P = 0.001$; treatment condition on ipsilateral side: $F[2,15] = 5.04$; $P = 0.02$). The significant effect of treatment condition was explained by the 0.01 nmol condition being significantly lower than saline ($P < 0.05$). NBOX also provided significant protection against NRHypo neurotoxicity (treatment condition × side interaction: $F[2,22] = 6.04$, $P = 0.008$; treatment condition on ipsilateral side: $F[2,22] = 3.74$; $P = 0.04$). The significant effect of treatment condition was explained by the 0.25 nmol condition being associated with fewer vacuolated neurons than the saline condition ($P < 0.05$). Rimcazole provided significant protection against NRHypo neurotoxicity (treatment condition × side interaction; $F[2,15] = 13.40$, $P = 0.0005$; treatment condition on ipsilateral side: $F[2,15] = 7.46$; $P = 0.006$). The significant effect of treatment condition was explained by the 50 nmol condition being associated with fewer vacuolated neurons than the saline condition ($P < 0.005$). In contrast clonidine, when injected into the RSC, did not alter the neurotoxicity (treatment condition × side interaction; $F[1,10] = 0.95$, $P = 0.35$). The numbers in parentheses indicate the number of animals in each condition. (e) Photograph of a whole

MK801 was omitted. Except for the cyclothiazide experiments, the total injected volume was 1.0 μl, regardless of the number of agents injected. For the cyclothiazide experiments cyclothiazide was injected separately in a volume of 1.0 μl. In these experiments, the brains were sectioned at several rostrocaudal levels to permit localization of the injection site and quantitative evaluation of the vacuole reaction in RSC. Counts of injured neurons were obtained from the section closest to the injection site.

Based upon previously obtained data, for each protective agent we hypothesized that a specific region mediated its protective effect—diagonal band for clonidine, RSC for scopolamine, NBQX and rimcazole. For each of these region-agent combinations, an ANOVA model with two independent factors, treatment condition and side, and one dependent measure, severity of neurotoxicity was used to evaluate the data. For those ANOVAs with a significant interaction term, the ANOVA was decomposed to determine if there was a significant effect of condition on the ipsilateral side. For those experiments that had multiple doses of the active compound, significant findings were further analyzed with planned comparisons to determine which dose was most likely to explain the finding. For each region-agent combination other regions were subsequently tested as controls in order to demonstrate the specificity of the finding. Anterior cingulate and isocortex lateral to the RSC were examined for rimcazole, scopolamine and NBQX. Medial septum, nucleus basalis, locus coeruleus, nucleus accumbens, RSC and anterior cingulate were examined for clonidine.

Previously we found that numerous antimuscarinic agents can block NRHypo neurotoxicity and we ranked these agents in order of their ED_{50} blocking potencies.[13] Five different molecular subtypes of the muscarinic receptor have been cloned and numerous antimuscarinic agents have been studied by Bolden and colleagues[38] for their *in vitro* binding affinities to each of the five muscarinic receptor subtypes. In order to explore which muscarinic receptor subtype may be involved in the neurotoxic reaction, we compared the order of potencies of antimuscarinic agents for blocking NMDA antagonist neurotoxicity with their order of binding affinities to each of the muscarinic receptor subtypes. To judge statistical significance of these comparisons, we used the conservative Spearman correlation coefficients test which does not make assumptions about the underlying data.

Materials

MK801, scopolamine HBr, NBQX, clonidine, carbachol, and cyclothiazide were purchased from Sigma-Aldrich (St Louis, MO, USA). Kainic acid (KA) and AMPA were purchased from RBI (St Louis, MO, USA). (+) SKF-10,047 was obtained from the NIDA Drug Safety System (Bethesda, MD, USA). Rimcazole was a gift from Burroughs Wellcome (Research Triangle Park, NC, USA).

Results

Receptor identification and localization experiments

Non-RSC localization of NMDA receptor To determine whether the NMDA receptors through which NMDA antagonist drugs trigger a neurotoxic reaction in RSC are located in the RSC, we injected the powerful NMDA antagonist, MK801 (0.5 nmoles and 5 nmoles) directly into the RSC. As Table 1 indicates, this did not cause neurotoxic changes in RSC neurons. Since this amount of MK801 should effectively block all NMDA receptors in the RSC, these findings indicate that systemic MK801 interacts with NMDA receptors in non-RSC brain regions to produce the RSC neurotoxic reaction. It must also be assumed that at least two neurons—the injured RSC neuron and an NMDA receptor-bearing neuron located outside of the RSC—are involved in this neurotoxic syndrome.

RSC localization of muscarinic receptor Previously we have demonstrated that a series of muscarinic antagonists, when administered systemically, prevent MK801 neurotoxicity, the most potent agent in this series being scopolamine.[13] In the present study we injected scopolamine directly into RSC and found that it dose dependently prevented the MK801-induced vacuole reaction in RSC neurons (Figures 2a–d). A dose of 0.01 nmol provided 67% blockade of the toxic reaction ipsilateral to the injection site ($P < 0.05$; Figure 2d). Injections of scopolamine into more distal cortical areas (adjacent isocortex ($n = 6$) and anterior cingulate ($n = 6$)) did not significantly reduce the toxic reaction on the ipsilateral compared to the contralateral side ($P > 0.25$). These observations suggest that hyperactivation of a muscarinic receptor in RSC is a

brain of a rat that received an injection of clonidine (1.0 nmol) into the vertical limb of the diagonal band of Broca immediately before being treated systemically with MK801 (0.5 mg kg^{-1}, sc). Vertically oriented lines indicate approximate level from which sections in (f) and (g) were taken. Numbers above lines indicate distance of lines from Bregma in mm. (f) Low power light micrograph of section located at 0.7 mm relative to Bregma. Arrows indicate track made by injection needle showing its placement into the vertical limb of the diagonal band of Broca. (g) Section of RSC cut at –5.5 mm from Bregma from the brain depicted in (e). This section was used for evaluating the severity of NRHypo neurotoxicity. The cortex from the corpus callosum (CC) to the white line is the part of the RSC that is susceptible to NRHypo neurotoxicity. (h) Bar graph illustrating the amount of protection against NRHypo neurotoxicity in RSC provided by the injection of clonidine (1.0 nmol) directly into the vertical limb of the diagonal band of Broca. Clonidine provided statistically significant protection against NRHypo neurotoxicity in the RSC ipsilateral to the injection site compared to that seen with saline (treatment condition × side interaction: $F[1,10] = 60.51$, $P < 0.0001$; treatment condition on ipsilateral side: $F[1,10] = 7.46$; $P = 0.02$). Clonidine also provided statistically significant protection ($P = 0.0001$) in the RSC on the side ipsilateral as compared to the side contralateral to the injection site. The numbers in parentheses indicate the number of animals in each condition.

Table 1 Inability to reproduce NRHypo neurotoxicity by RSC injection of specific agents

Agent(s) and dose	*n*	*Comments*
MK801		
0.5 nmoles	4	no vacuolated neurons
5 nmoles	4	no vacuolated neurons
Carbachol		
50 nmoles	4	excitotoxicity; an occasional vacuolated neuron
5 nmoles	5	no vacuolated neurons
AMPA		
1 nmole	4	excitotoxicity present to varying degrees
0.1 nmoles	4	no vacuolated neurons
KA		
1 nmole	4	excitotoxicity present to varying degrees
0.1 nmoles	4	no vacuolated neurons
(+)SKF-10,047		
100 nmoles	5	no vacuolated neurons
(+)SKF-10,047 + Carbachol		
50 nmoles + 5 nmoles	6	no vacuolated neurons
5 nmoles + 5 nmoles	5	no vacuolated neurons
(+)SKF-10,047 + KA		
25 nmoles + 1 nmole	4	excitotoxicity present to varying degrees
5 nmoles + 0.1 nmoles	4	no vacuolated neurons

critical factor in the mediation of NRHypo neurotoxicity. Consistent with this interpretation, we have found that systemically administered NMDA antagonists induce excessive release of ACh in RSC.[39]

Identification of the muscarinic receptor subtype Bolden *et al*[38] have evaluated the affinity of various muscarinic antagonists for binding to each of the five molecular subtypes of muscarinic receptors. We previously had evaluated the potencies of each of the same muscarinic antagonists for blocking NRHypo neurotoxicity.[13] Comparing the order of potencies of these muscarinic antagonists for blocking NRHypo neurotoxicity with their order of binding affinities, a significant correlation was found only for the m_3 receptor subtype ($r = 0.865$; $P < 0.05$; Table 2). Therefore, we tentatively propose that the cholinergic contribution to the neurotoxic mechanism may be mediated via the m_3 receptor subtype.

Table 2 Potency of antimuscarinics in preventing NRHypo neurotoxicity *vs* binding affinity to the five molecular subtypes of muscarinic receptors

Compound tested	*ED_{50} mg kg^{-1}, ip*[a]	*K_d in nM*[b]				
		m_1	*m_2*	*m_3*[c]	*m_4*	*m_5*
Scopolamine	0.13	1.1	2.0	0.44	0.8	2.07
Benztropine	1.29	0.23	1.4	1.1	1.1	2.8
Trihexyphenidyl	2.42	1.6	7	6.4	2.6	15.9
Atropine	2.67	0.5	0.9	1.1	0.6	1.7
Biperiden	3.70	0.48	6.3	3.9	2.4	6.3
Procyclidine	4.52	4.6	25	12.4	7	24
Diphenhydramine	17.54	100	120	229	112	260

[a] As reported by Olney *et al*.[13]
[b] As reported by Bolden *et al*.[38]
[c] $P < 0.05$, Spearman rank correlation coefficient, two-tailed.

RSC localization of non-NMDA glutamate receptor In addition to inducing vacuolization of RSC neurons, NMDA antagonists also trigger abnormal expression of heat shock protein (Kd 72) in the same RSC neurons,[13,40] both responses being apparently a manifestation of the same toxic process. Sharp and colleagues have found that DNQX, a non-NMDA receptor antagonist, when administered systemically, can block PCP-induced HSP-72 expression,[41] which suggests that a non-NMDA receptor could be involved in the neurotoxic reaction. We have confirmed this by two approaches: (1) to MK801-treated adult rats, we systemically administered NBQX, another antagonist of the non-NMDA receptor (30 mg kg^{-1}, sc × 3 doses), and found that it confers 75% protection against NRHypo neurotoxicity ($P = 0.02$; Figure 3). (2) We also injected NBQX directly into RSC and found that it produced a significant dose-dependent reduction in the vacuole reaction on the ipsilateral side. A dose of 0.25 nmoles suppressed the neurotoxic reaction on the ipsilateral side by 53% ($P < 0.05$, Figure 2d). Injecting NBQX into the anterior cingulate ($n = 7$) or adjacent lateral isocortex ($n = 6$) did not significantly influence the neurotoxic reaction in RSC ($P > 0.25$ for both locations), indicating that the non-NMDA receptor being blocked by NBQX is located in the RSC. These findings suggest that hyperactivation of a non-NMDA glutamate receptor in RSC is also a critical factor in the mediation of NRHypo neurotoxicity. Consistent with this interpretation is a recent report that NRHypo causes excitatory amino acid release in RSC.[42]

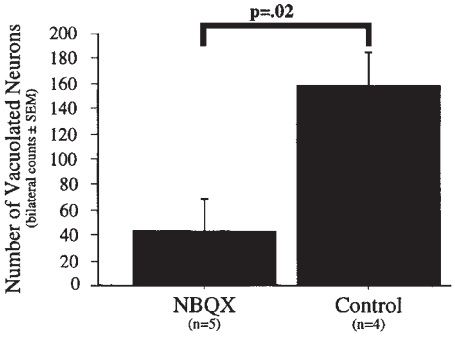

Figure 3 Ability of systemic NBQX to protect against NRHypo neurotoxicity. NBQX, when administered systemically (30 mg kg^{-1}, sc, × 3) to adult rats treated with MK801 0.5 mg kg^{-1}, sc, significantly decreased ($P = 0.02$) the number of vacuolated neurons in the RSC compared to animals treated with MK801 alone (control). Number in parentheses indicates the number of animals in each condition.

RSC localization of sigma site/receptor In prior experiments, rimcazole and DTG, both relatively selective but not very potent sigma ligands, blocked NRHypo neurotoxicity when injected systemically.[33] Another sigma ligand, (+)SKF-10,047, counteracted this blocking action. In the present experiments, when rimcazole was injected directly into the RSC, it dose-dependently suppressed the vacuole reaction ipsilateral to the injection (Figure 2d). A dose of 50 nmoles provided 76% blockade of the toxic reaction ipsilateral to the injection site ($P < 0.005$). Injections into anterior cingulate ($n = 6$) and adjacent lateral isocortex ($n = 7$) were ineffective ($P > 0.25$ for both locations) in blocking the RSC neurotoxic reaction. These findings indicate that the RSC is the site of action of rimcazole and suggest that a sigma recognition site in the RSC is involved in the circuitry that mediates NRHypo neurotoxicity.

Basal forebrain localization of α_2 adrenergic receptor Previous studies have shown that a series of α_2 adrenergic agonists, when administered systemically to adult rats, prevent the neurotoxic action of NMDA antagonists.[34] Moreover, in microdialysis studies we have demonstrated that the release of ACh in RSC induced by systemic MK801 is suppressed by systemic administration but not by direct intra-RSC injection of the α_2 adrenergic agonist, clonidine.[39] This latter result indicates that the α_2 adrenergic receptor with which clonidine interacts to suppress the release of ACh in RSC is not located within the RSC. Consistent with the microdialysis result, in the present study direct injection of clonidine into the RSC at a dose of 1 nmole ($n = 6$) did not provide neuroprotection (Figure 2d; $P = 0.35$) against MK801 neurotoxicity in RSC. If the α_2 adrenergic receptors are not located within the RSC, the most likely place where they might be located would be in the basal forebrain where the vast majority of cortical-projecting cholinergic cell bodies reside.[43,44] These cholinergic cell bodies are dispersed over a large area but tend to cluster within four major zones—medial septal nucleus, vertical limb of the diagonal band of Broca, horizontal limb of the diagonal band of Broca and the nucleus basalis. It is neurons in the vertical diagonal band zone that provide the majority of the cholinergic input to the RSC.[44] Consistent with this anatomical observation, injection of clonidine (1 nmole) into the vertical limb of the diagonal band blocked 33% of the damage ipsilateral to the injection ($P = 0.02$; Figures 2e–h). Injections of clonidine into the horizontal limb of the diagonal band ($n = 5$) provided less protection (24%; $P = 0.03$) consistent with the interpretation that this area provides a minor amount of cholinergic input to the RSC.[44] Injection of 1 nmole of clonidine into the medial septum ($n = 6$), which lies just superior to the vertical limb of the diagonal band, or injection into the nucleus basalis ($n = 4$) provided no protection ($P > 0.25$ for both). Injection into other areas was also ineffective (locus coeruleus ($n = 4$), nucleus accumbens ($n = 7$), anterior cingulate ($n = 5$); $P > 0.25$ for each site). These results indicate that the site of action of clonidine is in an area of brain that contains cholinergic cell bodies that project to the RSC.

Recreating NRHypo neurotoxicity by activating proximal receptor systems

Blockade of muscarinic, non-NMDA glutamatergic and sigma receptors in the RSC prevents MK801's neurotoxic action indicating that hyperactivation of each of these receptors may contribute to the mechanism by which NMDA antagonists injure RSC pyramidal neurons. However, it is unknown from these results whether hyperactivation of one, two or all three of these receptors would be sufficient for inducing the damage.

To determine if excessive activation of any one of these three receptors alone is sufficient to cause a vacuole reaction, we injected into RSC either the muscarinic agonist, carbachol, or a non-NMDA receptor agonist (either KA or AMPA), or (+) SKF-10,047. Neither carbachol, AMPA, KA, nor (+) SKF-10,047 reproduced NRHypo neurotoxicity (Table 1). These results suggest that hyperactivation of just one of these receptor systems in the RSC region is not sufficient to produce the neurotoxic reaction.

In light of evidence that NMDA antagonists, when given sc, cause an excessive cerebrocortical release of both ACh[39,45,46] and Glu,[47,48] we examined whether carbachol in combination with a non-NMDA Glu agonist (KA or AMPA) would reproduce the RSC neurotoxic reaction. The combined injection of carbachol and AMPA (5 nmoles + 1 nmole; or 5 nmoles + 0.1 nmoles) tended to produce either excitotoxic-like changes without the formation of vacuoles or no discernible pathology (Figure 4g and Figure 5). Injections of the combination of carbachol and KA (5 nmoles + 1 nmole) produced a mild vacuole reaction but excitotoxic changes (ie swollen dendritic processes) were also present (data not shown). Lowering the dose of KA to 0.3 nmoles in the carbachol-KA cocktail resulted in a mild vacuole reaction (Figure 5). Carbachol (5 nmoles)

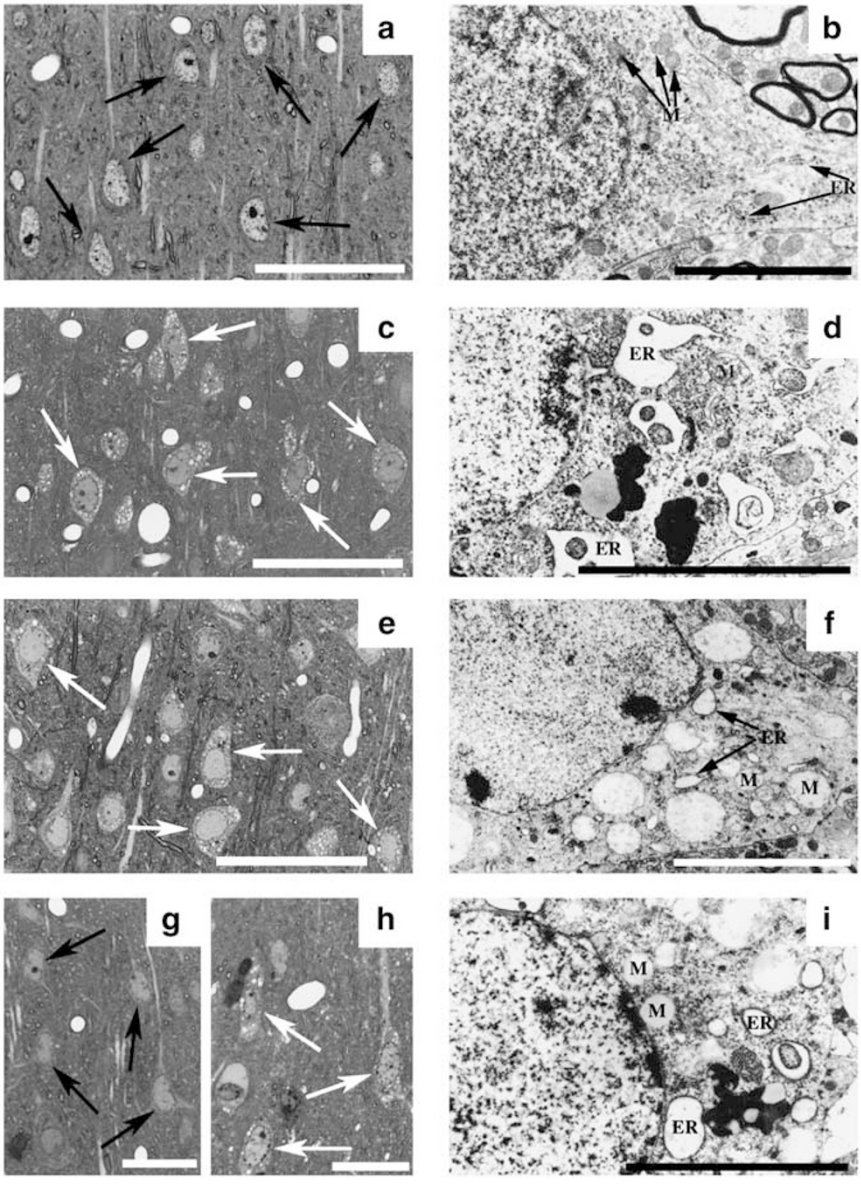

Figure 4 Recreation of NRHypo neurotoxicity by the injection of muscarinic and non-NMDA agonists. (a) Light micrograph taken from the RSC of a rat systemically injected with saline. Pyramidal neurons (black arrows) are normal in appearance. White scale bar = 50 μm. (b) Electronmicrograph (EM) of a pyramidal neuron from layer III–IV of the RSC of a saline-treated control animal. Mitochondria (M) and endoplasmic reticulum (ER) are normal. Black scale bar = 0.5 μm. (c) Light micrograph taken from the RSC of a rat injected systemically with MK801 (0.5 mg kg^{-1}, sc). Several pyramidal neurons (white arrows) are damaged and demonstrate hallmarks of NRHypo neurotoxicity (cytoplasmic vacuoles and swollen cytoplasm). White scale bar = 50 μm. (d) EM of a layer III–IV RSC pyramidal neuron from a rat treated with systemic MK801. Endoplasmic reticulum (ER) and mitochondria (M) are dilated. Black scale bar = 0.5 μm. (e) Light micrograph taken from the RSC of a rat injected into the RSC with carbachol (5 nmol) and KA (0.1 nmol). Several pyramidal neurons (white arrows) are damaged and demonstrate cytoplasmic vacuoles and swollen cytoplasm. These neurons are indistinguishable from those damaged by the systemic injection of MK801. White scale bar = 50 μm. (f) EM of a layer III–IV RSC pyramidal neuron from an animal treated with carbachol and KA. Endoplasmic reticulum (ER) and mitochondria (M) are dilated. White scale bar = 0.5 μm. (g) Light micrograph taken from the RSC of a rat injected with carbachol (5 nmol) and AMPA (0.1 nmol). The pyramidal neurons (black arrows) are normal in appearance and are indistinguishable from those seen in saline-treated animals. White scale bar = 25 μm. (h) Light micrograph taken from the RSC of a rat injected with carbachol (5 nmol), AMPA (0.1 nmol) and cyclothiazide (25 nmol). Several pyramidal neurons (white arrows) are damaged and demonstrate the hallmarks of NRHypo neurotoxicity. White scale bar = 25 μm. (i) EM of a layer III–IV RSC pyramidal neuron from an animal treated with carbachol, AMPA and cyclothiazide. Endoplasmic reticulum (ER) and mitochondria (M) show changes similar to those seen in (d) and (f). Black scale bar = 0.5 μm.

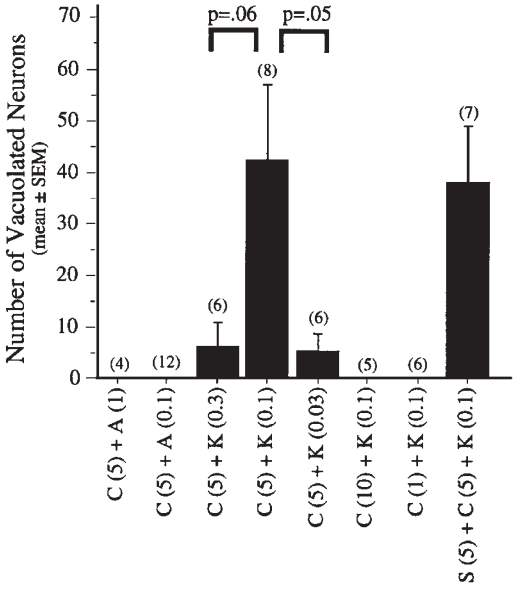

Figure 5 Quantification of the severity of NRHypo neurotoxicity recreated by the injection of different agents. Animals received an injection of several different cocktails and the number of vacuolated neurons were subsequently enumerated. Numbers in parentheses above each bar are the number of animals in that condition. Numbers in parentheses in the legend are the amount of each agent in nmoles injected into the RSC. Total volume for each condition was 1 μl. C = carbachol, A = AMPA, K = KA, S = (+)SKF-10,047.

combined within even lower dose of KA (0.1 nmoles) induced an apparently stronger vacuole reaction (P = 0.06) with only minimal to no excitotoxic-like changes (Figure 5). The histological changes produced by this combination occurred in layer III-IV pyramidal neurons (Figures 4e and 4f) and was similar in quality to the changes that occur when NMDA antagonists are injected systemically (Figures 4c and d). Further reductions in the amount of KA (0.03 nmoles) led to a diminution of severity of the neurotoxic reaction (P = 0.05; Figure 5). Maintaining the amount of KA at its optimum dose (0.1 nmole) while increasing carbachol to 10 nmoles or decreasing it to 1 nmole resulted in loss of the ability to produce the neurotoxicity (Figure 5).

The combined injection of (+)SKF-10,047 and carbachol or (+)SKF-10,047 and KA did not reliably reproduce the neurotoxic reaction (Table 1). Finally, the injection of all three agents (SKF-10,047 + carbachol + KA (5 nmoles + 5 nmoles + 0.1 nmoles)) did reproduce the neurotoxicity (Figure 5). The mean number of damaged neurons was not appreciably different from that seen with carbachol and KA (P = 0.8). Additional modifications of the amount of each agent did not produce a more robust vacuole response (data not shown). Thus, we conclude that hyperactivation of ACh and non-NMDA receptors on the RSC pyramidal neurons is probably the critical and final step in NRHypo neurotoxicity and that the sigma receptor may modify activity at either the ACh or non-NMDA receptor.

To determine whether the sigma receptor modifies the cholinergic or glutamatergic arm, either KA or carbachol was injected into the RSC of rats that had been treated with MK801 (0.5 mg kg^{-1}, sc) and a neuroprotective dose of the sigma antagonist, rimcazole (30 mg kg^{-1}, ip). If rimcazole affects the cholinergic arm, then unilateral injection of carbachol should override rimcazole's protective effect and allow the neurotoxicity to occur on the ipsilateral side, while the contralateral side would remain free from vacuoles, because rimcazole's action would still be operative on the contralateral side. A similar logic would apply to the KA injections. Carbachol-injected rats had significantly more neurotoxicity on the ipsilateral side *vs* the contralateral side (P = 0.02; Figure 6). In contrast the KA animals did not demonstrate any contra- ipsi-difference (P = 0.6; Figure 6). These findings suggest that rimcazole via a sigma site in the RSC may modulate the cholinergic arm of the circuit.

Thus, we conclude that simultaneous hyperactivation of both a muscarinic and non-NMDA Glu receptor on the same neuron results in NRHypo neurotoxicity. While the ability of carbachol+KA and not carbachol+AMPA to reproduce the neurotoxicity would seem to implicate a kianate receptor subtype, it is possible that KA produces the damage because AMPA receptors do not desensitize to KA but do desensitize to AMPA. To further explore the subtype of the non-NMDA receptor involved in NRHypo neurotoxicity, we examined whether cyclothiazide, which eliminates AMPA receptor desensitization, if added to the carbachol+AMPA cocktail, would enable the cocktail to produce NRHypo neurotoxicity. Adding cyclothiazide (75 nmoles (n = 9)) to the carbachol+AMPA (5 nmoles + 0.1 nmoles) cocktail resulted in an RSC neurotoxic reaction (Figures 4h and i), whereas this combination of carbachol and AMPA alone did not (Figure 4g). This result suggests that an AMPA preferring receptor might be involved in this neurotoxic syndrome.

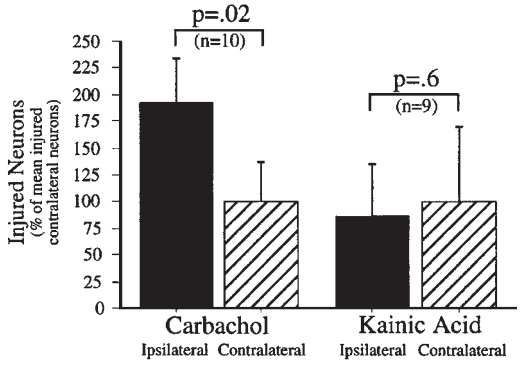

Figure 6 Carbachol's reversal of rimcazole's protection against NRHypo neurotoxicity. Animals were treated with MK801 (0.5 mg kg^{-1}, sc) and rimcazole (30 mg kg^{-1}, ip). In addition either carbachol (5 nmol, n = 10) or KA (0.1 nmol, n = 9) was directly injected into the RSC. Ipsilateral = side of brain that received the injection of carbachol or KA. Contralateral = side of brain that did not receive the injection.

Discussion

In prior studies involving systemic administration of pharmacological agents or microdialysis methods, significant clues had been gained regarding the general nature of the toxic process triggered by NMDA antagonists and the complex array of receptor mechanisms involved. Here we have explored in greater detail important aspects of the circuitry that mediates NRHypo neurotoxicity. The focus of our experiments was confined to the reversible reaction selectively affecting RSC neurons, the intention being to expand the focus to the more widespread irreversible reaction after gaining better insight into the reversible process.

In the present study, the first point addressed was whether a blocking action of an NMDA antagonist at NMDA receptors within the RSC is sufficient to trigger the RSC neurotoxic reaction, and the answer is clearly no. Although the neurotoxic reaction manifests selectively in neurons of the RSC, injection of the NMDA antagonist, MK801 directly into the RSC at doses calculated to block NMDA receptors throughout RSC did not reproduce the neurotoxic reaction. This signifies that it requires blockade of NMDA receptors at one or more sites outside the RSC to trigger this neurotoxic reaction.

Building on prior evidence that the RSC neurotoxic reaction can be blocked by systemic administration of antimuscarinic agents, we have now demonstrated that the RSC neurotoxic reaction can also be blocked by injecting the cholinergic muscarinic antagonist directly into the RSC, but not by injecting it into other nearby regions. Together with microdialysis evidence that systemic MK801 causes a sustained massive release of ACh in the RSC region, this finding supports the interpretation that hyperactivation of a muscarinic receptor plays an important role in mediating the neurotoxic reaction.

Based on a comparison of *in vitro* receptor binding affinities and *in vivo* neuroprotective potencies of muscarinic receptor antagonists, we tentatively conclude that it is the m_3 subtype of muscarinic receptor that mediates the neurotoxic reaction. This type of analysis is fraught with potential error due to the many variables that are different between the two types of data being compared. Several pharmacokinetic variables (eg different rates of penetration of blood–brain barriers and different elimination half-lives) will affect the *in vivo* neuroprotective potency data but not the *in vitro* receptor binding data. However, until new agonists and antagonists are developed that are highly selective for each muscarinic receptor subtype, this is the best approach available.

Based on recent evidence that NRHypo causes excitatory amino acid release in RSC,[42] and on our present finding that either systemic administration or direct intra-RSC injection of NBQX, a non-NMDA Glu receptor antagonist, blocks the RSC neurotoxic reaction induced by MK801, we propose that hyperactivation of a non-NMDA Glu receptor on the RSC neuron may be an additional critical component of the mechanism that mediates this neurotoxic reaction.

From the above information, it would be predicted that direct injection into the RSC of an agent that activates muscarinic receptors and/or an agent that activates non-NMDA glutamate receptors would locally reproduce NRHypo neurotoxicity. The present experiments revealed that direct RSC injection of either a muscarinic receptor agonist or a non-NMDA glutamate receptor agonist alone was not sufficient to reproduce the neurotoxic reaction. Since the simplest explanation for this result would be that it required hyperactivation of both a muscarinic and non-NMDA Glu receptor on the same neuron to reproduce the neurotoxic reaction in that neuron, we injected cocktails containing various amounts of a muscarinic agonist (carbachol) and a non-NMDA Glu agonist (AMPA or KA) and found that the reaction was faithfully reproduced by a combination of carbachol and KA, but not by carbachol and AMPA. While this would seem to implicate a KA-receptor subtype, one cannot be confident in this conclusion for the following reasons: (1) AMPA and KA receptor ligands show a very high degree of receptor cross-reactivity; (2) when AMPA or KA are applied continuously to AMPA-preferring receptors, a desensitization mechanism is triggered in response to AMPA but not to KA.[49,50] For further clarification, we used cyclothiazide, an agent that is known to prevent AMPA receptor desensitization. Consistent with a role for an AMPA preferring receptor, we found that the addition of cyclothiazide to the carbachol+AMPA cocktail allowed the neurotoxic reaction to be expressed. It will be important to test new antagonists, when available, that show a high degree of specificity for AMPA *vs* KA receptor subtypes in order to determine whether AMPA receptors, KA receptors, or both are involved.

Any of several classes of agents that either mimic or potentiate the action of GABA at $GABA_A$ receptors, when administered systemically, are effective in protecting against NRHypo neurotoxicity, with benzodiazepines being less effective than barbiturates or GABAergic anesthetics.[13,37,51] This implies that $GABA_A$ receptors play a very important role. While the present experiments were not designed to identify the specific loci in the brain where the critical $GABA_A$ receptor interactions occur, the functional role(s) of the $GABA_A$ receptor must include regulation of the activity of cholinergic neurons because in microdialysis experiments we have shown that drugs that activate $GABA_A$ receptors prevent or reverse the action of MK801 in releasing ACh in the RSC.[39] We tentatively postulate that the locus of integration between GABAergic and cholinergic neurons is in the diagonal band region of the basal forebrain in that it is well established that there are clusters of GABAergic neurons that co-localize with and modulate the activity of magnocellular cholinergic neurons in all subregions of the basal forebrain.

Consistent with the above findings, we propose that the mechanism of NRHypo neurotoxicity is indirect, and involves a polysynaptic chain of events whereby blockade of NMDA receptors in multiple non-RSC brain regions results in excessive release of both ACh and glutamate at muscarinic (probably m_3) and

AMPA/KA receptors, respectively, on RSC neurons. We propose that simultaneous excessive stimulation of RSC neurons by these two excitatory transmitters is the proximal mechanism by which neuronal injury occurs. As Figure 7 illustrates, the most likely type of wiring to explain how blockade of excitatory (NMDA) transmission can lead to release of excessive activity in both cholinergic and non-NMDA glutamatergic excitatory pathways, would be if the NMDA receptors are located on inhibitory neurons, and through these NMDA receptors glutamate tonically drives the inhibitory neurons, thereby maintaining tonic inhibitory restraint over the two excitatory pathways.

While the proposed circuitry is specific for the reversible neurotoxicity in the RSC, a similar disinhibition mechanism might underlie the more widespread degeneration seen in other corticolimbic brain regions

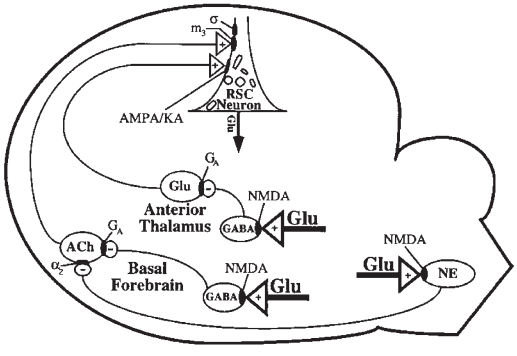

Figure 7 Circuitry proposed to mediate NRHypo neurotoxicity. To explain NRHypo neurotoxicity in the RSC, we propose that Glu, acting through NMDA receptors on GABAergic and noradrenergic neurons, maintains tonic inhibitory control over two major excitatory pathways that convergently innervate RSC neurons. Systemic administration of an NMDA antagonist would block NMDA receptors, thereby abolishing inhibitory control over both of the excitatory inputs to the RSC neuron. The disinhibited excitatory pathways then would simultaneously hyperactivate the RSC neuron, which would create chaotic disruption of multiple intracellular signaling systems, thereby causing immediate derangement of cognitive functions subserved by the afflicted neurons (psychotomimetic effects), and reversible or irreversible neuronal injury, depending on how long the disruption lasts. Although our present findings do not clarify the origin of the glutamatergic cell bodies that project to the AMPA/KA receptors in the RSC, we postulate these cell bodies are located in the anterior thalamus because RSC is known to receive a dense glutamatergic projection from the anterior thalamus[73] and MK801-induced HSP72 expression in RSC can be prevented by injections of muscimol into the anterior thalamus.[74] This circuit diagram focuses exclusively on RSC neurons. We hypothesize that a similar disinhibition mechanism and similar but not necessarily identical neural circuits and receptor mechanisms mediate damage induced in other corticolimbic brain regions by sustained NRHypo. (+) = excitatory input; (–) = inhibitory input; ACh = acetylcholine; NE = norepinephrine; Glu = glutamate; GABA = γ-amino butyric acid; α_2 = α_2 subtype of adrenergic receptor; G_A = $GABA_A$ subtype of GABA receptor; m_3 = m_3 subtype of muscarinic cholinergic receptor; AMPA/KA = AMPA/KA subtype of Glu receptor; NMDA = NMDA subtype of Glu receptor; σ = sigma site.

with more prolonged periods of NRHypo. Consistent with this proposal NMDA antagonists produce excessive release of Glu[42,47,48,52] and ACh[45,46,53,54] in multiple corticolimbic regions. The excessive release of neurotransmitters and subsequent overexcitation also might underlie the ability of NMDA antagonists (eg MK801, ketamine and PCP) to increase metabolism in several corticolimbic regions.[55–68]

If the NRHypo circuitry is present in several corticolimbic regions, why then does the reversible neurotoxicity appear only in the RSC and not in the other regions? Because the dose response curve for inducing the neurotoxicity in the RSC is an inverted U-shape (Figure 5), we suspect that the selectivity of the reversible neurotoxicity for the RSC is a function of the relative stimulation of the muscarinic *vs* non-NMDA receptor. Thus, other regions might not develop the reversible neurotoxicity because in these other regions there might be too much or too little Glu release compared to ACh release. The result would be the inability to produce the reversible neurotoxicity in these other regions with a low dose of an NMDA antagonist. However, higher doses would produce a severe enough disinhibitory state that irreversible degeneration would occur. Another possibility is that there could be subtle differences in the circuit. It will be important to explore these possibilities in future studies.

Previously we have found that systemically administered α_2 adrenergic agonists, including clonidine, protect against NRHypo neurotoxicity[34] and can reverse the excessive release of Ach induced in RSC by NMDA antagonists.[39] However, because clonidine was not effective in reversing the excessive ACh release when it was directly injected into the RSC, we reasoned that clonidine suppresses ACh release in RSC by interacting with α_2 adrenergic receptors on cholinergic elements outside of the RSC. An additional implication would be that the axon terminals given rise to ACh release do not originate from cholinergic neurons within RSC. Our present findings support the interpretation that clonidine's protective activity against the RSC neurotoxic reaction is due to an action in the diagonal band region of the basal forebrain where cholinergic cell bodies, which project to the RSC, are concentrated. We propose, therefore, that noradrenergic neurons in the locus coeruleus send axonal projections that terminate on α_2 adrenergic receptors on basal forebrain cholinergic neurons, and modulate the activity of these neurons (Figure 7).

The exact role of sigma receptors remains unclear, but based on our present findings we propose that sigma receptor ligands influence NRHypo neurotoxicity by interacting in the RSC region with the cholinergic limb of the circuit. This interpretation is consistent with evidence that sigma ligands inhibit the stimulatory effects of cholinergic agents, including carbachol, on PI turnover.[69] Thus, in our model we have tentatively placed the sigma site adjacent to the muscarinic receptor (Figure 7). Other features of the proposed circuitry (eg, location of $5HT_{2A}$ receptors and location

of glutamatergic cell bodies that project to the AMPA/KA receptor) remain to be elucidated.

Our findings contribute to an improved understanding of the mechanisms by which an NRHypo state can result in neurotoxicity and psychosis. These findings, therefore, may have practical significance in relation to disease processes putatively mediated by an NRHypo mechanism (eg, Alzheimer's disease, and idiopathic psychotic disorders). While a detailed discussion of the putative role of an NRHypo mechanism in these disease processes is beyond the scope of the present writing, this information has recently been reviewed.[70–72] Improved understanding of the circuitry underlying NRHypo neurotoxicity may also provide insights into methods for preventing the psychotomimetic and neurotoxic actions of NMDA antagonist drugs, and thereby allow the considerable therapeutic potential of these drugs to be realized.

Acknowledgments

Supported by AG11355, DA05072, SDAC DA00290 (NBF) from NIH, and a Young Investigator Award (NBF) from NARSAD.

References

1 Domino EF, Luby ED. Abnormal mental states induced by phencyclidine as a model of schizophrenia. In: Domino EF (ed). *PCP (Phenyclidine): Historical and Current Perspectives*. NPP Books: Ann Arbor, 1981, pp 401–418.
2 Javitt DC, Zukin SR. Recent advances in the phenyclidine model of schizophrenia. *Am J Psychiatry* 1991; **148**: 1301–1308.
3 Kristensen JD, Svensson B, Gordh T. The NMDA receptor-antagonist CPP abolishes neurogenic 'wind-up pain' after intrathecal administration in humans. *Pain* 1992; **51**: 249–253.
4 Herrling P. D-CPPene (SDZ EAA 494), a competitive NMDA antagonist. Results from animal models and first results in humans. *Neuropsychopharmacology* 1994; **10**: (**3S/Part 1**): 591S.
5 Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD *et al*. Subanesthetic effects of the noncompetitive NMDA antagonist, Ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* 1994; **51**: 199–214.
6 Grotta J, Clark W, Coull B, Pettigrew LC, Mackay B, Goldstein LB *et al*. Safety and tolerability of the glutamate antagonist CGS 19755 (Selfotel) in patients with acute ischemic stroke. Results of a phase IIa randomized trial. *Stroke* 1995; **26**: 602–605.
7 Lahti AC, Koffel B, LaPorte DJ, Tamminga CA. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology* 1995; **13**: 9–19.
8 Malhotra AK, Pinals DA, Weingartner H, Sirocco K, Missar CD, Pickar D *et al*. NMDA receptor function and human cognition: the effects of ketamine in healthy volunteers. *Neuropsychopharmacology* 1996; **14**: 301–307.
9 Muir KW, Grosset DG, Lees KR. Effects of prolonged infusion of the NMDA antagonist aptiganel hydrochloride (CNS 1102) in normal volunteers. *Clin Neuropharmacol* 1997; **20**: 311–321.
10 Newcomer JW, Farber NB, Jevtovic-Todorovic V, Selke G, Kelly Melson A, Hershey T *et al*. Ketamine-induced NMDA receptor hypofunction as a model of memory impairment in schizophrenia. *Neuropsychopharmacology* 1999: **20**: 106–118.
11 Olney JW, Labruyere J, Price MT. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science* 1989; **244**: 1360–1362.
12 Allen HL, Iversen LL. Phencyclidine, dizocilpine, and cerebrocortical neurons. *Science* 1990; **247**: 221.
13 Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA. NMDA antagonist neurotoxicity: mechanism and prevention. *Science* 1991; **254**: 1515–1518.
14 Fix AS, Horn JW, Wightman KA, Johnson CA, Long GG, Storts RW *et al*. Neuronal vacuolization and necrosis induced by the noncompetive N-methyl-D-aspartate (NMDA) antagonist MK(+)801 (dizocilpine maleate): a light and electron microscopic evaluation of the rat retrosplenial cortex. *Exp Neurol* 1993; **123**: 204–215.
15 Fix AS, Wozniak DF, Truex LL, McEwen M, Miller JP, Olney JW. Quantitative analysis of factors influencing neuronal necrosis induced by MK-801 in the rat posterior cingulate/retrosplenial cortex. *Brain Res* 1995; **696**: 194–204.
16 Ellison G, Switzer RC. Dissimilar patterns of degeneration in brain following four different addictive stimulants. *Neuroreport* 1993; **5**: 17–20.
17 Ellison G. Competitive and non-competitive NMDA antagonists induce similar limbic degeneration. *Neuroreport* 1994; **5**: 2688–2692.
18 Corso TD, Sesma MA, Tenkova TI, Der TC, Wozniak DF, Farber NB *et al*. Multifocal brain damage induced by phencyclidine is augmented by pilocarpine. *Brain Res* 1997; **752**: 1–14.
19 Horvath ZC, Czopf J, Buzsaki G. MK-801-induced neuronal damage in rats. *Brain Res* 1997; **753**: 181–195.
20 Jevtovic-Todorovic V, Todorvic SM, Mennerick S, Powell S, Dikranian K, Benshoff N *et al*. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. *Nature Med*: 1998; **4**: 460–463.
21 Wozniak DF, Dikranian K, Ishimaru M, Nardi A, Corso TD, Tenkova TI *et al*. Disseminated corticolimbic neuronal degeneration induced in rat brain by MK-801: potential relevance to Alzheimer's disease. *Neurobiol Dis* 1998; **5**: 305–322.
22 Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* 1995; **52**: 998–1007.
23 Anand A, Charney DS, Oren DA, Berman RM, Hu XS, Cappiello A *et al*. Attenuation of the neuropsychiatric effects of ketamine with lamotrigine. *Arch Gen Psychiatry* 2000; **57**: 270–276.
24 Gonzales RA, Brown LM, Jones TW, Trent RD, Westbrook SL, Leslie SW. N-methyl-D-aspartate mediated responses decrease with age in Fischer 344 rat brain. *Neurobiol Aging* 1991; **12**: 219–225.
25 Wenk GL, Walker LC, Price DL, Cork LC. Loss of NMDA, but not GABA-A, binding in the brais of aged rats and monkeys. *Neurobiol Aging* 1991; **12**: 93–98.
26 Magnusson KR, Cotman CW. Age-related changes in excitatory amino acid receptors in two mouse strains. *Neurobiol Aging* 1993; **14**: 197–206.
27 Saransaari P, Oja SS. Dizocilpine binding to cerebral cortical membranes from developing and ageing mice. *Mech Ageing Dev* 1995; **85**: 171–181.
28 Ulas J, Cotman CW. Decreased expression of N-methyl-D-aspartate receptor 1 messenger RNA in select regions of Alzheimer brain. *Neuroscience* 1997; **79**: 973–982.
29 Olney JW, Farber NB. Excitatory transmitter neurotoxicity and Alzheimer's disease. In: Giacobini E, Becker R (eds). *Alzheimer Disease: Therapeutic Stategies*. Birkhauser: Boston, 1994, pp 293–298.
30 Olney JW, Wozniak DF, Farber NB. Excitotoxic neurodegeneration in Alzheimer disease; new hypothesis and new therapeutic strategies. *Arch Neurol* 1997; **54**: 1234–1240.
31 Farber NB, Newcomer JW, Olney JW. Glutamatergic transmission: therapeutic prospects for schizophrenia and Alzheimer's disease. In: Egebjerg J, Schousboe A, Krogsgaard-Larsen P (eds). *Glutamate and GABA Receptors and Transporters: Structure, Function and Pharmacology*. Taylor and Francis: London (in press).
32 Vogt BA. Structural organization of cingulate cortex: areas, neurons, and somatodendritic transmiter receptors. In: Vogt BA, Gabriel M (eds). *Neurobiology of Cingulate Cortex and Limbic Thalamus*. Birkhauser: Boston, 1993, pp 19–70.
33 Farber NB, Price MT, Labruyere J, Nemnich J, St Peter H, Wozniak DF *et al*.. Antipsychotic drugs block phencyclidine receptor-mediated neurotoxicity. *Biol Psychiatry* 1993; **34**: 119–121.
34 Farber NB, Foster J, Duhan NL, Olney JW. α_2 adrenergic agonists prevent MK-801 neurotoxicity. *Neuropsychopharmacology* 1995; **12**: 347–349.

35 Farber NB, Hanslick J, Kirby C, McWilliams L, Olney JW. Serotonergic agents that activate $5HT_{2A}$ receptors prevent NMDA antagonist neurotoxicity. *Neuropsychopharmacology* 1998; **18**: 57–62.
36 Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*, 4th edn. Academic Press: New York, 1998.
37 Jevtovic-Todorovic V, Kirby CO, Olney JW. Isoflurane and propofol block neurotoxicity caused by MK-801 in the rat posterior cingulate/retrosplenial cortex. *J Cereb Blood Flow Metab* 1997; **17**: 168–174.
38 Bolden C, Cusack B, Richelson E. Antagonism by antimuscarinic and neuroleptic compounds at the five cloned human muscarinic cholinergic receptors expressed in Chinese hamster ovary cells. *J Pharmacol Exp Ther* 1992; **260**: 576–580.
39 Kim SH, Price MT, Olney JW, Farber NB. Excessive cerebrocortical release of acetylcholine induced by NMDA antagonists is reduced by GABAergic and α_2-adrenergic agonists. *Mol Psychiatry* 1999; **4**: 344–352.
40 Sharp FR, Jasper P, Hall J, Noble L, Sagar SM. MK-801 and ketamine induce heat shock protein HSP72 in injured neurons in posterior cingulate and retrosplenial cortex. *Ann Neurol* 1991; **30**: 801–809.
41 Sharp JW, Petersen DL, Langford MT. DNQX inhibits phencyclidine (PCP) and ketamine induction of the hsp70 heat shock gene in the rat cingulate and retrosplenial cortex. *Brain Res* 1995; **687**: 114–124.
42 Noguchi K, Johnson R, Ellison G. The effects of MK-801 on aspartate and glutamate levels in the anterior cingulate and retrosplenial cortices: an in vivo microdialysis study. *Soc Neurosci Abs* 1998; **24**: 233.
43 Mesulam MM, Mufson EJ, Levey AI, Wainer BH. Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J Comp Neurol* 1983; **214**: 170–197.
44 Woolf NJ. Cholinergic systems in mammalian brain and spinal cord. *Prog Neurobiol* 1991; **37**: 475–524.
45 Giovannini MG, Camilli F, Mundula A, Pepeu G. Glutamatergic regulation of acetylcholine output in different brain regions: a microdialysis study in the rat. *Neurochem Int* 1994; **25**: 23–26.
46 Hasegawa M, Kinoshita H, Amano M, Hasegawa T, Kameyama T, Nabeshima T. MK-801 increases endogenous acetylcholine release in the rat parietal cortex: a study using brain microdialysis. *Neurosci Lett* 1993; **150**: 53–56.
47 Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* 1997; **17**: 2921–2927.
48 Adams B, Moghaddam B. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci* 1998; **18**: 5545–5554.
49 Sommer B, Keinanen K, Verdoorn TA, Wisden W, Burnashev N, Herb A *et al*.. Flip and flop: a cell-specific functional switch in glutamate-operated channels of the CNS. *Science* 1990; **249**: 1580–1585.
50 Patneau DK, Vyklicky L, Mayer ML. Hippocampal neurons exhibit cyclothiazide-sensitive rapidly desensitizing responses to kainate. *J Neurosci* 1993; **13**: 3496–3509.
51 Ishimaru M, Fukamauchi F, Olney JW. Halothane prevents MK-801 neurotoxicity in the rat cingulate cortex. *Neurosci Lett* 1995; **193**: 1–4.
52 Moghaddam B, Adams B. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. *Science* 1998; **281**: 1349–1352.
53 Giovannini MG, Mutolo D, Bianchi L, Michelassi A, Pepeu G. NMDA receptor antagonists decrease GABA outflow from the septum and increase acetylcholine outflow from the hippocampus: a microdialysis study. *J Neurosci* 1994; **14**: 1358–1365.
54 Giovannini MG, Giovannelli L, Bianchi L, Kalfin R, Pepeu G. Glutamatergic modulation of cortical acetylcholine release in the rat: a combined in vivo microdialysis, retrograde tracing and immunohistochemical study. *Eur J Neurosci* 1997; **9**: 1678–1689.
55 Hawkins R, Hass WK, Ransoff J. Measurement of regional glucose utilization in vivo using [2-^{14}C] glucose. *Stroke* 1979; **10**: 690–703.
56 Meibach RC, Glicks D, Cox R, Maayani S. Localisation of phencyclidine-induced changes in brain energy metabolism. *Nature* 1979; **282**: 625–626.
57 Nelsom SR, Howard RB, Cross RS, Samson F. Ketamine-induced changes in regional glucose utilization in the rat brain. *Anesthesiology* 1980; **52**: 330–334.
58 Lund JP, Miller JJ, Courville J. [^{3}H]2-deoxyglucose capture in the hippocampus and dentate gyrus of ketamine-anesthetized rat. *Neurosci Lett* 1981; **24**: 149–153.
59 Crosby G, Crane AM, Sokoloff L. Local changes in cerebral glucose utilization during ketamine anesthesia. *Anesthesiology* 1982; **56**: 437–443.
60 Oguchi K, Arakawa K, Nelson SR, Samson F. The influence of droperidol, diazepam and physostigmine on ketamine-induced behavior and brain regional glucose utilization in rat. *Anesthesiology* 1982; **57**: 353–358.
61 Hammer RP, Herkenham M. Altered metabolic activity in the cerebral cortex of rats exposed to ketamine. *J Comp Neurol* 1983; **220**: 396–404.
62 Davis DW, Mans AM, Biebuyck JF, Hawkins RA. The influence of ketamine on regional brain glucose use. *Anesthesiology* 1988; **69**: 199–205.
63 Kurumaji A, McCulloch J. Effects of MK-801 upon local cerebral glucose utilisation in conscious rats and in rats anaesthetised with halothane. *J Cereb Blood Flow Metab* 1989; **9**: 786–794.
64 Clow DW, Lee SJ, Hammer RP. Competitive (AP7) and non-competitive (MK-801) NMDA receptor antagonists differentially alter glucose utilization in rat cortex. *Synapse* 1991; **7**: 260–268.
65 Boddeke HW, Wiederhold KH, Palacios JM. Intracerebroventricular application of competitive and non-competitive NMDA antagonists induce similar effects upon rat hippocampal electroencephalogram and local cerebral glucose utilization. *Brain Res* 1992; **585**: 177–183.
66 Gao XM, Shirakawa O, Du F, Tamminga CA. Delayed regional metabolic actions of phencyclidine. *Eur J Pharmacol* 1993; **241**: 7–15.
67 Sharkey J, Ritchie IM, Butcher SP, Kelly JS. Comparison of the patterns of altered cerebral glucose utilisation produced by competitive and non-competitive NMDA receptor antagonists. *Brain Res* 1996; **735**: 67–82.
68 Duncan GE, Moy SS, Knapp DJ, Mueller RA, Breese GR. Metabolic mapping of the rat brain after subanesthetic doses of ketamine: potential relevance to schizophrenia. *Brain Res* 1998; **787**: 181–190.
69 Walker JM, Bowen WD, Walker FO, Matsumoto RR, De Costa B, Rice KC. Sigma receptors: biology and function. *Pharmacol Rev* 1990; **42**: 355–402.
70 Farber NB, Newcomer JW, Olney JW. The glutamate synapse in neuropsychiatric disorders: focus on schizophrenia and Alzheimer's disease. In: Ottersen OP, Langmoen I, Gjerstad L (eds). *The Glutamate Synapse as a Therapeutical Target: Molecular Organization and Pathology of the Glutamate Synapse*. Elsevier: Amsterdam, 1998, pp 421–437.
71 Farber NB, Newcomer JW, Olney JW. Glycine agonists: what can they teach us about schizophrenia? *Arch Gen Psychiatry* 1999; **56**: 13–17.
72 Farber NB, Newcomer JW. The role of NMDA receptor hypofunction in idiopathic psychotic disorders. In: Geller B, Delbello M (eds). *Child and Early Adolescent Bipolar Disorder*. Guilord Publications: New York (in press).
73 van Groen T, Vogt BA, Wyss JM. Interconnections between the thalamus and retrosplenial cortex in the rodent brain. In: Vogt BA, Gabriel M (eds). *Neurobiology of Cingulate Cortex and Limbic Thalamus*. Birkhauser: Boston, 1993, pp 123–150.
74 Tomitaka S, Tomitaka M, Tolliver BK, Sharp FR. Heat shock protein HSP72 induced in rat retrosplenial cortex by dizocilpine is caused by the thalamo-cortical neurons in anterior thalamus. *Soc Neurosci Abs* 1999; **25**: 2234.