CNS Drug Reviews
Vol. 7, No. 2, pp. 172–198

Gacyclidine: A New Neuroprotective Agent Acting at the N-Methyl-D-Aspartate Receptor

Hélène Hirbec*, Manuel Gaviria†, and Jacques Vignon*

**INSERM U336, DPVSN, Institut de Biologie, Montpellier, France;*
†Centre PROPARA, Parc Euromédecine, Montpellier, France.

Key Words: Gacyclidine—Gacyclidine enantiomers—GK11—NMDA-receptor antagonist—Neuroprotection — Neurotoxicity — Neurotrauma — Organophosphorous poisoning — Non-NMDA binding sites.

ABSTRACT

Gacyclidine is a new phencyclidine derivative with neuroprotective properties. Tritiated gacyclidine and its enantiomers bind to NMDA receptors with binding parameters similar to those of other non-competitive NMDA receptor antagonists. The (–)enantiomer, (–)GK11, exhibits an affinity (2.5 nM) similar to that of dizocilpine (MK-801), while the (+)enantiomer, (+)GK11, has a 10 times lower affinity. When its interaction with NMDA receptors is prevented, gacyclidine binds also to "non-NMDA" binding sites which are mainly located in the molecular layer of the cerebellum on the dendritic tree of Purkinje cells. These binding sites do not appear to be related to any known neurotransmitters.

In primary cortical cultures, gacyclidine and its enantiomers, at 0.1 to 5.0 μM, prevent glutamate-induced neuronal death. In rats, *in vivo* neurotoxicity of gacyclidine is far low than that of MK-801. No necrotic neurons were detected in animals sacrificed at 18 or 96 h after treatment with gacyclidine (1, 5, 10 or 20 mg/kg i.v.). At the highest (20 mg/kg) but not the lower doses (1–100 mg/kg) electron microscopy revealed the presence of few cytoplasmic or intramitochondrial vacuoles. In soman-treated monkeys gacyclidine enhanced neuroprotective activity of "three drugs cocktail" (atropine + diazepam + pralidoxime). Moreover, in rats, gacyclidine exerts a dose- and time-dependent neuroprotection in three models of spinal cord lesion. Beneficial effects of gacyclidine include reduction of lesion size and improvement of functional parameters after injury. In traumatic brain injury models gacyclidine improves also behavioral parameters and

Address correspondence and reprint requests to: Dr. Jacques Vignon, INSERM U336, DPVSN, Institut de Biologie, 4 Boulevard Henri IV, F-34060 Montpellier France.
Tel: +33 (4) 67.60.11.23. Fax: +33 (4) 67.54.06.10. E-mail: vignon@cit.enscm.fr

neuronal survival. Optimal protection is obtained when gacyclidine is administered at 0 to 30 min after injury.

It is, therefore, concluded that gacyclidine exhibits neuroprotective effects similar to those of other NMDA receptor antagonists, with the advantage of being substantially less neurotoxic maybe due to its interaction with "non-NMDA" binding sites.

INTRODUCTION

The ability of glutamate exposure to trigger central neuronal death has been recognized for more than 40 years (60,81,84,90). Over the past two decades evidence implicating excitotoxicity in various central nervous system (CNS) diseases, including ischemia, anoxia, hypoglycemia, and traumatic injury, has accumulated (5,22,23,109,16). Excitotoxicity-induced neurodegeneration is a complex phenomenon; excessive release of glutamate leads to over-stimulation of its different receptors and, consequently, to the induction of intracellular metabolic cascades, which leads to delayed cell death involving both necrotic and apoptotic mechanisms (Fig. 1). This second step is highly dependent upon a local massive increase of the intracellular calcium concentration. The ionotropic N-methyl-D-aspartate (NMDA) glutamate receptors are highly permeable to Ca^{2+} and are, therefore, implicated in the early events leading to neuronal death (87,72). In *in vitro* cell culture models, selective blockade of NMDA receptors (NMDARs) prevents calcium influx and cell death due to glutamate exposure (12,17,18,69,97). Accrodingly, it has been shown *in vivo* that NMDAR antagonists have beneficial effects against CNS injury (58,68,101). As a result of this, great efforts have been devoted to the development of NMDAR antagonists to test in clinical trials (4).

The NMDAR is a complex pharmacological entity. Multiple agents modulate its function, and this complexity is increased by its molecular diversity (13,71,75,43). Different promising therapeutic strategies involving NMDAR antagonists have been investigated in clinical trials.

NMDAR antagonists are classified as competitive antagonists, which act at the glutamate site (AP5, CGS19755*), the glycine site (7-chlorokynurenate, HA966), or the polyamine site (ifenprodil, eliprodil). Other agents, belonging to various chemical series, can block the ion channel and behave as noncompetitive antagonists. These agents include dizocilpine (MK-801), some benzomorphans like SKF-10047 or cyclazocine, and phencyclidine (PCP) and PCP-like drugs such as ketamine and TCP (1-[1-(2-thienyl)cyclohexyl]piperidine).

Studies performed by Olney et al during the late 1980s have introduced some doubts about the feasibility of therapies based upon NMDAR antagonists. Indeed, it has been shown that, in healthy rodents, MK-801 and PCP may induce neuronal toxicity at therapeutic doses (82,85,86). Similar results were observed with competitive antagonists (19). NMDAR antagonists cause neurotoxic side effects consisting of pathomorphological changes at the level of the multipolar and pyramidal neurons of layers III and IV of the posterior cingulate and retrosplenial cortices (85,86,19). Two to 4 hours after treatment with NMDAR antagonists, electron microscopic evaluation of the affected neurons, dis-

* CGS19755 is (±)-cis-4-phosphonomethyl-2-piperidine carboxylic acid.

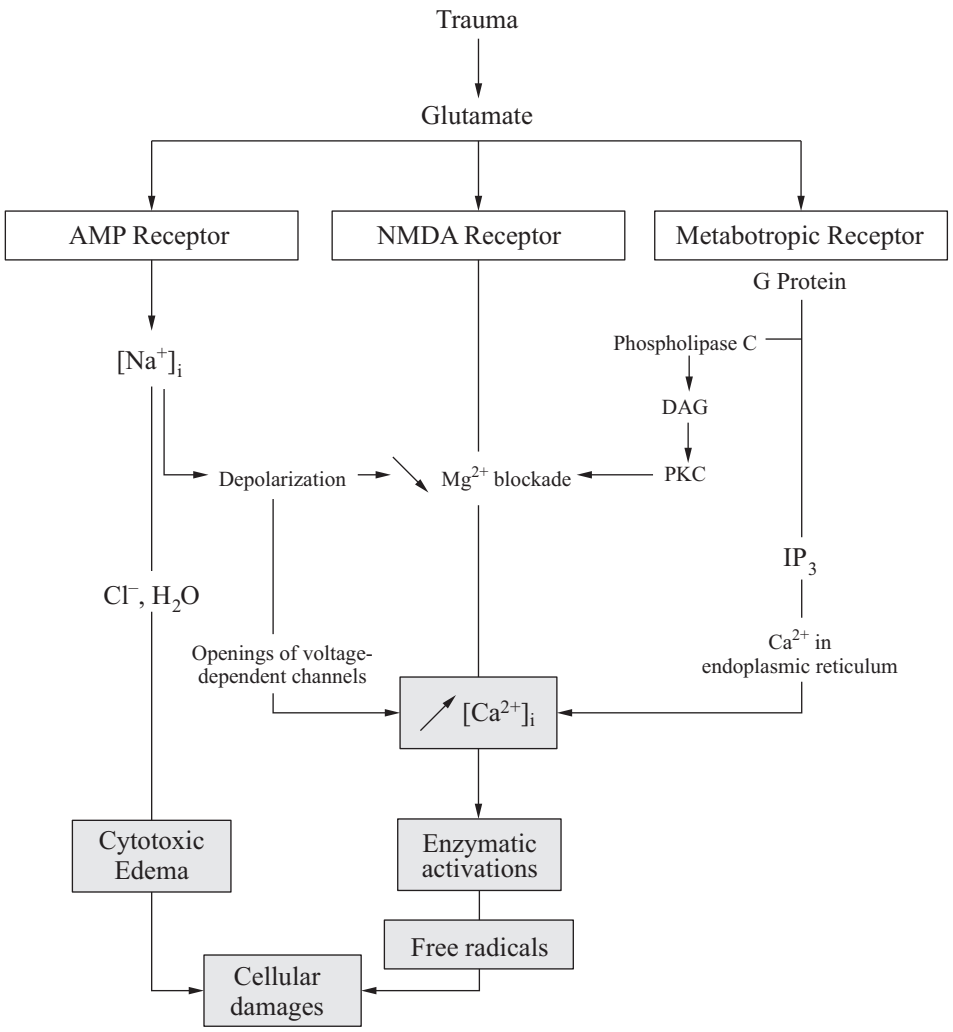

Fig. 1. The excitotoxic cascade.

played the formation of multiple vacuoles of heterogeneous size in the cytoplasmic compartments. These deleterious effects may occur after a single injection of therapeutic doses of an NMDAR antagonist, with chronic administration of NMDAR antagonists leading to more extensive neurotoxicity (19). At low doses, the vacuolization may be reversible, but higher doses can cause irreversible neuronal necrosis (2,27). To date, the pathogenesis of vacuolization and necrosis has not been definitively characterized; however, electron microscope findings support a role for compromised energy metabolism in affected neurons. Indeed, vacuole formation involving mitochondria and the endoplasmic reticulum was similar to that seen in hippocampal neurons after a hypoxic-ischemic episode. These deleterious effects were attributed to an excessive blockade of glutamatergic pathways that, in turn, induced excessive cholinergic functions (86). Therefore, the beneficial effect of

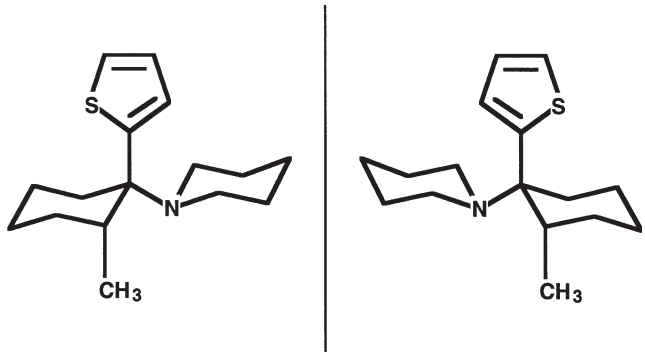

Fig. 2. Chemical structures of gacyclidine and its enantiomers, (+)GK11 and (–)GK11.

NMDAR antagonists could be hampered by their potential toxicity, which raises the question of whether the potential benefits of therapies involving NMDAR antagonists are sufficient to outweigh their risks. While the assumption of a higher beneficial effect versus risk is not unreasonable, it is not sufficient to dismiss the signal provided by the "Olney-type lesion" as irrelevant. Therefore, the American Department of Health and Human Services recommended, according to its 1990 Peripheral and Central Nervous Advisory Committee, that any new product claimed to be an NMDAR antagonist must be tested for neuronal vacuolization and/or necrosis in rats. They also recommended regularly including MK-801 as an internal positive control.

Our laboratory has been involved for many years in the study and the development of PCP and its derivatives. Previously, we have shown that TCP exhibits interesting neuroprotective properties (17,100), and in this review we report the pharmacological characteristics of the TCP derivative gacyclidine. Results obtained from this compound have shown that it is a potent neuroprotective agent and that it may offer advantages relative to other noncompetitive NMDAR antagonists.

CHEMISTRY

Gacyclidine [cis(pip/me)]-1-[1-(2-thienyl)-2-methylcyclohexylpiperidine] (Fig. 2) is a PCP derivative bearing a 2-thienyl group as an aromatic moiety and a methyl group at carbon 2. The methyl group introduces a chiral center and, therefore, gacyclidine is a racemate of two enantiomers, (+)GK11 [(+)-(1*R*-2*S*)-1-[1-(2-thienyl)-2-methylcyclohexylpiperidine] and (–)GK11 [(–)-(1*S*-2*R*)-1-[1-(2-thienyl)-2-methylcyclohexylpiperidine] (Fig. 2). Gacyclidine ($C_{16}H_{25}NS \cdot HCl$) is a white powder that is soluble in water (10^{-2} M). It is synthesized by the azide route according to the scheme presented in Fig. 3 (32). The enantiomers were resolved by a crystallization procedure via the diastereoisomeric salts formed with (+)- and (–)-di-O,O′-4-toluoyltartaric acid (10). The absolute configuration was determined by X-ray crystallography on a (–)GK11/HCl crystal (69). This process has been developed to an industrial level with the possibility of obtaining racemic resolution in the earliest stages via crystallization of quinine salts or enzymatic hydrolysis of an ester intermediate (10).

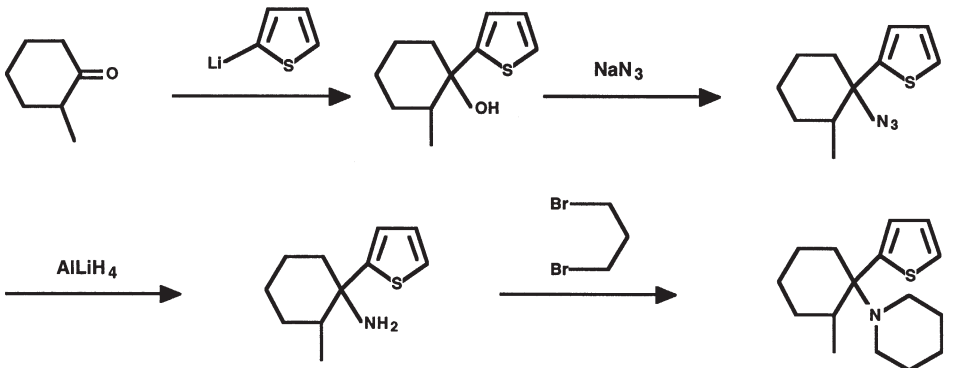

Fig. 3. Synthesis of gacyclidine.

PHARMACOLOGY

Receptor Binding Studies

Gacyclidine was first shown to exhibit a low affinity for muscarinic (12 μM) and opiate receptors (5 μM) (112); however, its affinity for the [^{3}H]PCP receptor (40 nM) was more closely correlated with its potency in the rotarod test (3.5 mg/kg) (113). Further studies have demonstrated that gacyclidine was the most potent PCP derivative at the PCP receptor (69). The separation of its enantiomers permitted us to demonstrate that the (–)enantiomer (4.3 nM) was as potent as MK-801 and that the (+)enantiomer was about 5 times less potent (20.4 nM) (36,41).

PCP and its analogs are potent inhibitors of dopamine uptake (110). For PCP derivatives bearing a phenyl group, a correlation has been shown between dopamine uptake and binding to the PCP receptor. The incorporation of a 2-thienyl group instead of the phenyl moiety leads to selectivity towards the PCP receptor. Conversely, the replacement of the phenyl group by a benzothiophenyl group increases the selectivity for the dopamine uptake site (11). Like TCP (IC_{50} = 1.3 μM), gacyclidine is a poor dopamine uptake inhibitor (IC_{50} = 7.9 μM) that exhibits a high selectivity for the PCP receptor.

The ability of gacyclidine and its enantiomers to inhibit the binding of specific radioligands to 43 neurotransmitter receptors has been investigated. Of these, gacyclidine and its enantiomers were found only to interact with the M_1 and M_2 muscarinic receptors (submicromolar affinities) and D_2 dopaminergic receptors (Table 1, Beaufour-Ipsen Company, internal report). It should be noted that no interaction was found with glutamatergic AMPA, kainate, or σ_1 and σ_2 receptors. The affinities determined for NMDARs with [^{3}H]MK-801 as a radioligand were identical to those measured using [^{3}H]TCP.

Interaction of [^{3}H]Gacyclidine and (+) and (–)[^{3}H]GK11 with NMDARs

The binding properties of [^{3}H]gacyclidine in the rat central nervous system (CNS) and other tissues have been investigated (38). Since it is a racemate, the binding characteristics of the two enantiomers have also been determined in parallel (41).

Using washed membrane preparations, the binding of noncompetitive NMDAR antagonists within the channel was shown to be highly dependent on the channel's opening state and to require the simultaneous presence of glutamate and glycine. On washed membranes, NMDAR agonists (i.e., glutamate, glycine, and spermine) stimulated gacyclidine binding. This effect was more pronounced on forebrain homogenates than on cerebellum or spinal cord homogenates and was inhibited by NMDAR antagonists such as AP5, CPP, and CGS19755 or kynurenate and 7-chlorokynurenate. It should be noted that, under control conditions, the competitive antagonists induced a partial decrease of basal binding, which indicates that residual glutamate and glycine were still present in the homogenates. Subcellular fractionation indicated that the binding is mainly associated with the synaptosomal fraction.

In the presence of glutamate and glycine, gacyclidine's binding characteristics differ somewhat from those of other noncompetitive NMDAR antagonists. Slow association and dissociation processes characterize the binding parameters of NMDAR antagonists, and they take several hours to reach equilibrium. With the enantiomers, these processes are multiphasic, which suggests they interact with multiple binding sites. This was confirmed by analysis of binding at equilibrium, which revealed that the (–)enantiomer interacts with two binding sites in forebrain homogenates. The (–)enantiomer exhibits an affinity of 2.5 nM, which is about eight and 12 times higher than those of the racemate (23.7 nM) and (+)GK11 (31.5 nM), respectively. Depending on the regions under consideration, the number of binding sites was similar to those detected with other noncompetitive NMDAR antagonists, although the binding parameters were different. High affinities, in the low nanomolar range, were found in forebrain and spinal cord homogenates. Lower affinities were found in the cerebellum. It is likely that these results reflect the different molecular composition and distribution of NMDARs. Indeed, NR_{2a} and NR_{2b} subunits are predominantly expressed in telencephalic regions, whereas the NR_{2c} subunit is found in the cerebellum (91,92,114). Accordingly, the regional distribution of [^{3}H]gacyclidine binding sites in the nervous system (Fig. 4) was in agreement with that of NMDARs, as determined either by autoradiographic (34,35,46,62,74,105,111) or by immunohistochemical (91,92) methods. The binding levels are high in the hippocampus and the cortex, intermediate in the striatum, and low in thalamic nuclei. In the cerebellum, the labeling intensity was lower, confirming that gacyclidine has low affinity in this region. Labeling was mainly concentrated in the granular layer. It is worth noting that the labeling ratio between the granular and molecular layers was lower than that reported for [^{3}H]MK-801 (105). In the spinal cord, the labeling is low and only minimal differences were observed among the cervical, thoracic, and lumbar levels. Globally, the binding is higher in the outer lamina (I to III) of the dorsal horn. In this tissue, the labeling patterns observed with the two enantiomers were slightly different: (–)[^{3}H]GK11 labeling was more discrete, whereas that of the (+)enantiomer was more uniform.

Pharmacological Characterization of (–) and (+)[^{3}H]GK11 Binding

The results of competition experiments performed with both enantiomers (Table 1) were generally better described by an interaction with two binding sites. In forebrain homogenates, the experimental conditions had to be modified to effectively show low-affinity binding sites. The affinities of MK-801, TCP, and gacyclidine and its enantiomers, as determined on high-affinity sites, were correlated with those determined with

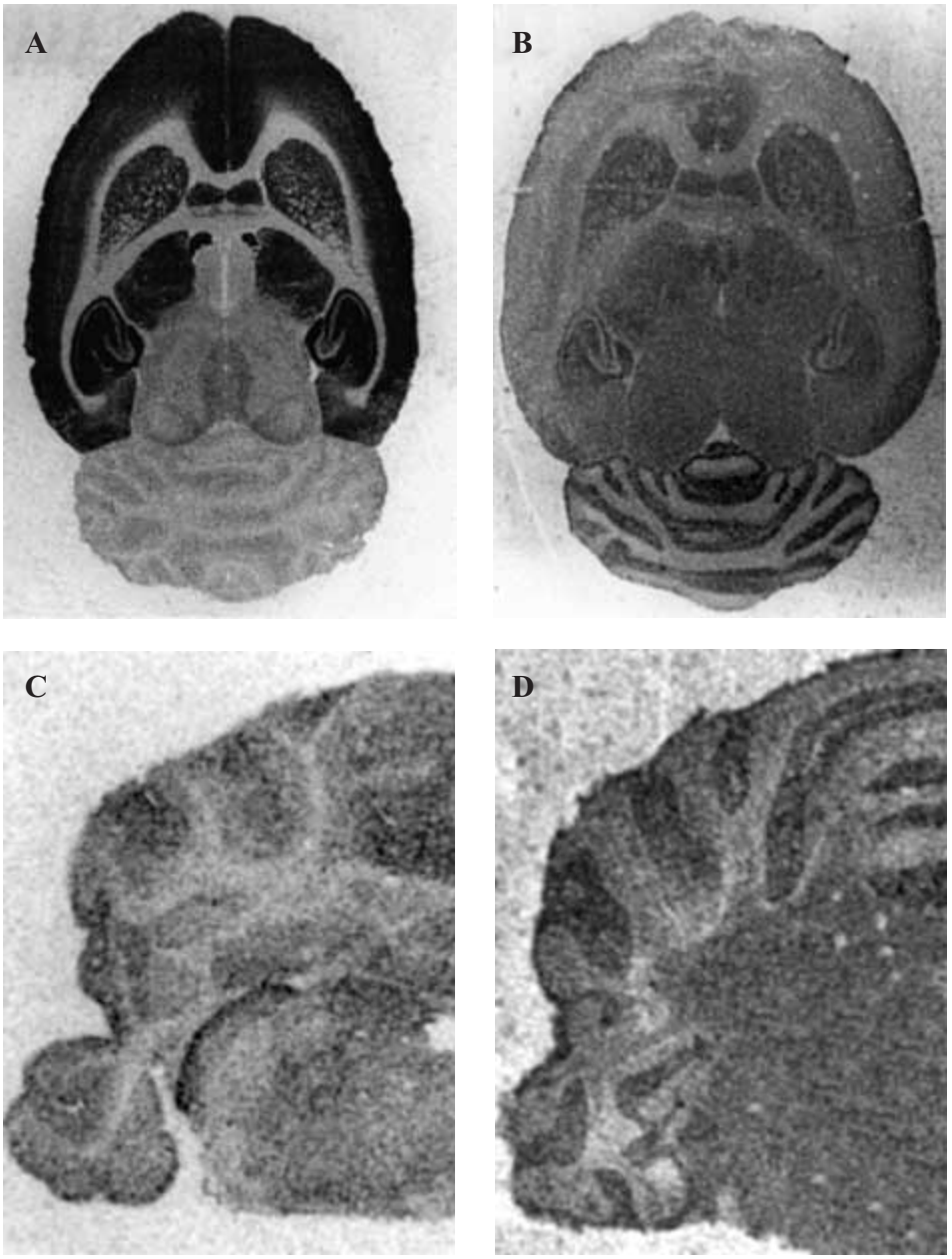

Fig. 4. Regional distribution of [^{3}H]gacyclidine binding sites in the rat brain. **A.** Binding of gacyclidine (5 nM) to NMDARs in the presence of glutamate and glycine. Note the low level of binding in the cerebellum. **C.** [^{3}H]gacyclidine (20 nM) binding in the cerebellum. **B** and **D**. Binding to non-NMDARs ([^{3}H]gacyclidine 20 nM).

[^{3}H]MK-801 or [^{3}H]TCP and represent those for NMDARs (36). (–)GK11 exhibited an affinity similar to that of MK-801.

TABLE 1. Affinities (K_i, nM) of gacyclidine and its enantiomers with NMDA, muscarinic, and dopaminergic receptors (Beaufour-Ipsen, personal communication)

Receptor	Gacyclidine	(–)GK11	(+)GK11
NMDA ([^{3}H]MK-801)	6.8	4.6	43
Muscarinic M_1	275	151	2790
Muscarinic M_2	864	587	>1000
Dopaminergic D_2	7200	>10,000	>10,000

No interaction was found with AMPA and kainate, α_1- and α_2-, β_1- and β_2-adrenergic, 5-HT (1 to 4), benzodiazepine, opiate (μ, δ, κ), CCK (A and B), histamine (H_1 and H_2), D_1, bradykinin, GRP, neuromedin, neurokinin, neurotensin, PACAP (I and II), PYY (Y_1 and Y_2) somatostatin (1 to 4) and TRH receptors, and calcium channels.

Interaction of [^{3}H]Gacyclidine, (+) and (–)[^{3}H]GK11 with "non-NMDA" Binding Sites

On low-affinity sites, MK-801 and TCP exhibited a low affinity (Table 2, second site). The data collected on forebrain and cerebellum homogenates with (–)[^{3}H]GK11 and (+)[^{3}H]GK11 were not identical (Table 1), suggesting that both enantiomers could bind to different low-affinity sites. In contrast to [^{3}H]TCP, (+) and (–)[^{3}H]GK11 binding to low-affinity sites was not inhibited by σ ligands.

In order to simplify data analysis, preparations where the binding of the radioligands to NMDARs was prevented were used for the latter study (40). Tissue homogenates or slices were extensively washed in the presence of a competitive NMDAR antagonist (CGS19755 10^{-5} μM). Binding and autoradiography experiments were performed after preincubation of the membranes and slices in the presence of CGS19755 100 μM. This procedure eliminated glutamate and glycine and maintained the ion channel in a closed conformation. Under such experimental conditions, a residual binding could be measured accounting for 10 to 15% and 60 to 70% of the control binding in forebrain and cerebellum homogenates, respectively. On autoradiographic films, the labeling pattern was considerably modified compared with incubations in the presence of glutamate and glycine (Fig. 4) (40). A low and relatively uniform specific binding was measured in telencephalic regions. In the cerebellum, the molecular layer was more densely labeled than under control conditions (i.e., in the presence of glutamate and glycine), and labeling of the granular and white matter layers was of the same intensity. In the granular layer, the labeling intensity of (+)[^{3}H]GK11 remained at the same level, whereas it had slightly decreased with (–)[^{3}H]GK11. These results led us to determine the effects of MK-801, under control conditions (i.e., in the presence of glutamate and glycine), on (–)[^{3}H]GK11 binding distribution in the cerebellum. Inclusion of MK-801 (1 μM) in the incubation medium almost completely inhibited the specific binding in the granular layer, but the binding level was unaffected in the molecular layer. Taken together, these results indicate that, when binding to NMDARs is prevented, gacyclidine binds to non-NMDA binding sites. These binding sites are abundant in the molecular layer of the cerebellum but can also be detected in the granular layer and in telencephalic regions. The increased binding in the molecular layer after the washing steps is likely to reflect the elimination of an endogenous factor that modulates gacyclidine binding to these sites (39).

TABLE 2. Inhibition of radioligand binding by unlabeled drugs in different CNS regions

A. Forebrain

	(–)[^{3}H]GK11 (2 nM)				(+)[^{3}H]GK11 (5 nM)			
	$\%_1$	K_{i1} (nM)	$\%_2$	K_{i2} (nM)	$\%_1$	K_{i1} (nM)	$\%_2$	K_{i2} (nM)
(+)GK11		31.5	–	–	82.0	18.8	13.0	1800
(–)GK11		2.7	–	–	92.0	2.2	6.2	8140
Gacyclidine		23.7	–	–	81.1	14.1	17.3	233
MK-801		2.4	–	–	89.5	2.0	9.4	>10 μM
TCP		10.9	–	–	89.1	8.5	6.0	>10 μM
	(–)[^{3}H]GK11 (20 nM)				(+)[^{3}H]GK11 (20 nM)			
	$\%_1$	K_{i1} (nM)	$\%_2$	K_{i2} (nM)	$\%_1$	K_{i1} (nM)	$\%_2$	K_{i2} (nM)
(+)GK11	78.6*	36.7*	20.1	29.9	74.5	13.8	26.8	403
(–)GK11	75.1	1.4	28.6	159	94.9	2.6	5.9	>10 μM
MK-801	94.2	2.0	9.1	5890	87.1	2.2	19.0	>10 μM
TCP	–	14.5	–	–	87.2	10.4	10.8	>10 μM

B. Cerebellum

	(–)[^{3}H]GK11 (2 nM)				(+)[^{3}H]GK11 (5 nM)			
	$\%_1$	K_{i1} (nM)	$\%_2$	K_{i2} (nM)	$\%_1$	K_{i1} (nM)	$\%_2$	K_{i2} (nM)
(+)GK11	83.8	54.6	13.6	2460		149.7		
(–)GK11	75.5	6.1	25.7	754	41.6	3.4	58.3	733
Gacyclidine	60.8	30.4	39.1	514	58.8	21.7	36.2	2215
MK-801	76.0	7.2	22.9	>10 μM	41.4	6.9	55.3	>10 μM
TCP	79.7	27.0	18.0	3319	41.1	19.1	54.8	5856
PRE-084	90.8	>100 μM	9.6	23.2	54.2	>100 μM	43.7	242
BD1063		>100 μM				>100 μM		
BD1008	80.9	52.6 μM	19.8	5.7	64.0	>100 μM	30.7	431
JH 68	72.3	314	26.6	6640	47.0	228	49.8	14.2 μM
JH6	86.8	3638	16.4	34.0	72.0	2419	26.0	>100 μM
CNS 1102	91.8*	28.3	9.0	2504	57.6	22.0	39.8	1042
Memantine	79.9*	652	17.3*	>100 μM	60.0	1016	41.0*	>100 μM

C. Spinal cord

	(–)[^{3}H]GK11 (2.5 nM)				(+)[^{3}H]GK11 (10 nM)			
	$\%_1$	K_{i1} (nM)	$\%_2$	K_{i2} (nM)	$\%_1$	K_{i1} (nM)	$\%_2$	K_{i2} (nM)
(+)GK11	45.3	5.1	59.0	204	22.2	2.6	78.6	188
(–)GK11	70.9	2.4	34.1	165	46.9	3.1	57.1	224
Gacyclidine	59.3	9.3	40.3	288	61.4	9.4	39.5	649
MK-801	80.6	2.2	18.0	2210	68.3	15.0	26.6	3560
TCP	62.1	6.7	38.8	158	21.6	4.7	76.9	229

Homogenates were incubated in the presence of increasing concentration of the test compounds with (–)[^{3}H]GK11 or (+)[^{3}H]GK11. The mean of at least three independent experiments was analyzed according to a single or a two-site model. When the two-site model is statistically more probable (Fisher's bilateral test), the proportion of high- and low-affinity sites are expressed as $\%_1$ and $\%_2$ and the corresponding K_i (expressed in nM) values as K_{i1} and K_{i2}, otherwise only K_i is indicated.

In order to determine which cell population non-NMDA binding sites are located on, autoradiography experiments were performed on brain slices from animals bearing mutations leading to selective cell loss in the cerebellum. Relative to control animals, PCD mice lacking Purkinje cells showed only residual binding, which was almost identical to that measured in the granular layer. In X-ray irradiated rats, which lack granular cells, the residual binding was identical to that measured in the molecular layer of control animals. These results indicate that the binding to non-NMDA binding sites in the molecular layer is probably associated with the dendritic tree of Purkinje cells (39).

PHARMACOLOGICAL STUDIES

Neuroprotection *In Vitro*

Primary cultures of embryonic cortical cells were used to test the neuroprotective effects of gacyclidine as well as those of (–) and (+)GK11 (18). In this study, the neuroprotective effects of gacyclidine and its enantiomers were also compared with those of the reference drug MK-801.

Drian et al. (18) have shown that neuroprotection against the *in vitro* neurotoxicity of 500 μM glutamate can be achieved by gacyclidine and its enantiomers at concentrations of 0.1 to 5 μM (Fig. 5). The neuroprotective efficacy of the (–)enantiomer is superior to that of the (+)enantiomer. However, the efficacy of the (+)enantiomer was not significantly different from that of the racemate. This study, therefore, confirms the potency of PCP derivatives to protect neurons against *in vitro* glutamate toxicity (17,100). It also supports the widely held view that neuroprotection can be afforded by NMDAR antagonists (12,22,97). Indeed, it should be noted that the *in vitro* neuroprotective efficacies of (–)GK11, (+)GK11, and MK-801 were perfectly correlated with their affinities for the NMDAR as measured with forebrain homogenates (41,69). The efficacy of the racemate, however, did not seem to be correlated with its affinity for NMDARs since the latter is close to that of (+)GK11, whereas its neuroprotective potency is close to that of (–)GK11 (18, 41,69). This apparent discrepancy may be explained by the fact that (–) and (+)GK11 might present complementary pharmacological properties (36). Therefore, as both enantiomers are present in the racemate, they may have synergistic effects. However, Michaud et al. (69) have shown, using [^{3}H]TCP as an NMDAR marker, that the affinity of gacyclidine and (–)GK11 are very similar. Whatever the reason for this difference, Drian et al. (18) have shown that the neuroprotective potency of gacyclidine is equal to that of MK-801. Moreover, examination of GABA immunoreactive cells in protected cultures (Fig. 6) has permitted Drian et al. to collect qualitative data concerning the surviving neurons. In particular, the comparison of cultures protected by 0.1 μM of MK-801 or (–)GK11 revealed that, with the former, GABA positive neurons displayed some suffering signs (personal communication). These cellular alterations were characterized by a retraction of the neurites and sometimes a moderate shrinkage of the neurons. In any event, the two enantiomers did not exhibit any qualitative differential neuroprotective characteristics; neither the size of the surviving cells nor their phenotype (GABA staining) was different for the compounds.

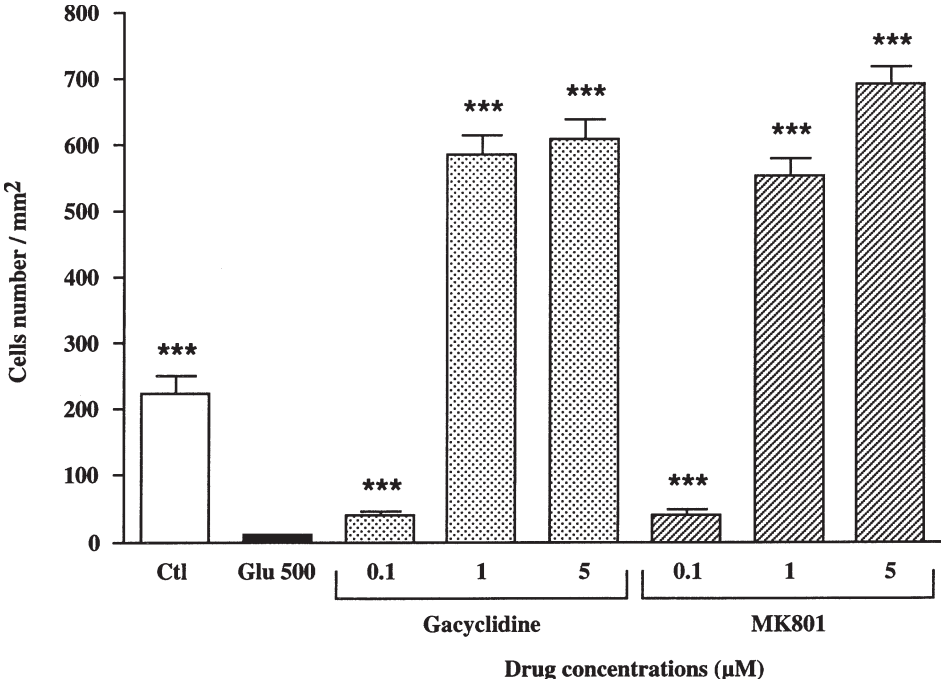

Fig. 5. Comparison of the effects of gacyclidine and MK-801 against glutamate toxicity on 18 days cortical cell primary culture. NSE-positive cells were counted 24 h after glutamate treatment. Cells were incubated for 5 min in a MEM-glucose medium in the presence of the appropriate drug. The medium was then replaced for 5 min by a solution containing glutamate (500 µM) and the drug. After exposure, the medium was replaced by a MEM-glucose medium for 24 h. Ctl = control culture; *** significantly different from glutamate 500 µM (Glu 500), $P < 0.001$. Higher values in treated cultures vs. controls are due to the protection against the deleterious effects of repeated medium changes. With permission from Drian et al. (22).

Other studies have also shown that a single 10-min treatment of cultured rat cortical neurons with gacyclidine (5 µM) prevented spontaneous neuronal death over long periods in culture (up to 73 days) (17). In similar experiments, Levallois et al. (57) have also shown that the same treatment improved the long-term survival of cultured human fetal spinal cord GABA-positive neurons. Such treatment, however, had some effects on the morphological appearance of GABAergic neurons. Indeed, in treated cultures, the extent of the neuritic field of large GABAergic neurons was significantly reduced.

Neurotoxicity *In Vivo*

As stated in the introduction, Olney's work has shown that in healthy rodents NMDAR antagonists can induce neurotoxicity whose intensity is dependent on both the dose and the treatment duration. In a study conducted to evaluate the neurotoxic effects of gacyclidine, Sandillon and Privat (personal communication) compared gacyclidine and its enantiomers with MK-801 and CNS-1102. Each drug was tested at doses of 1, 5, 10, and 20 mg/kg (the highest dose of MK-801, however, could not be tested because of high toxicity). With MK-801 at doses of 5 and 10 mg/kg, the animals exhibited abnormal behavior

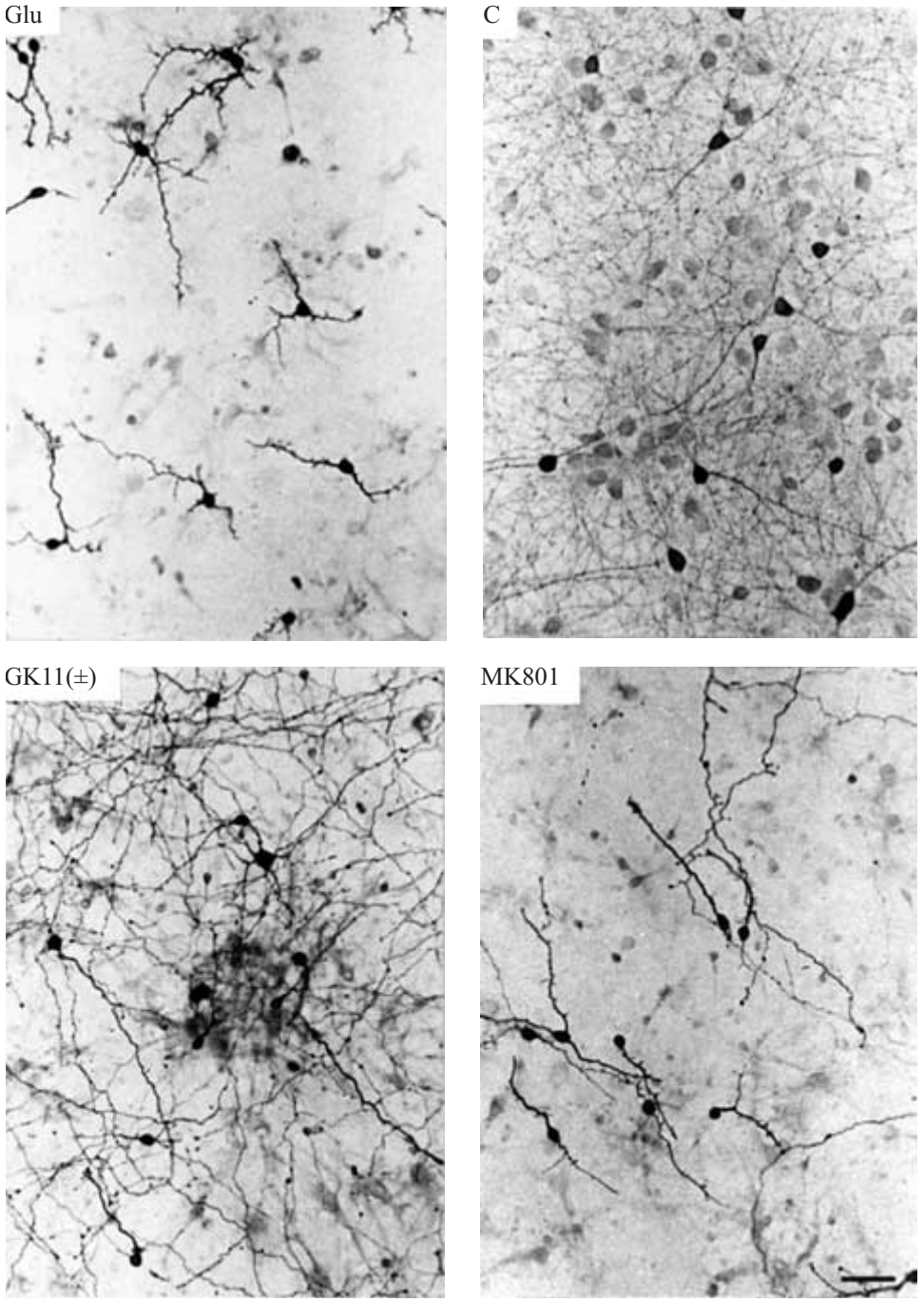

Fig. 6. Protection against glutamate toxicity by gacyclidine on GABAergic neurons. After glutamate treatment (Glu), most GABAergic neurons appear stunted, with small perikarya and spiky processes, contrasting with the control (C). When protected with 5 μM gacyclidine [GK11(±)], cell size and neurite appearance were not affected. When protected by 5 μM MK-801 (MK801), many GABAergic neurons showed stunted neurites. Scale bar = 40 μm. With permission from Drian et al. (22).

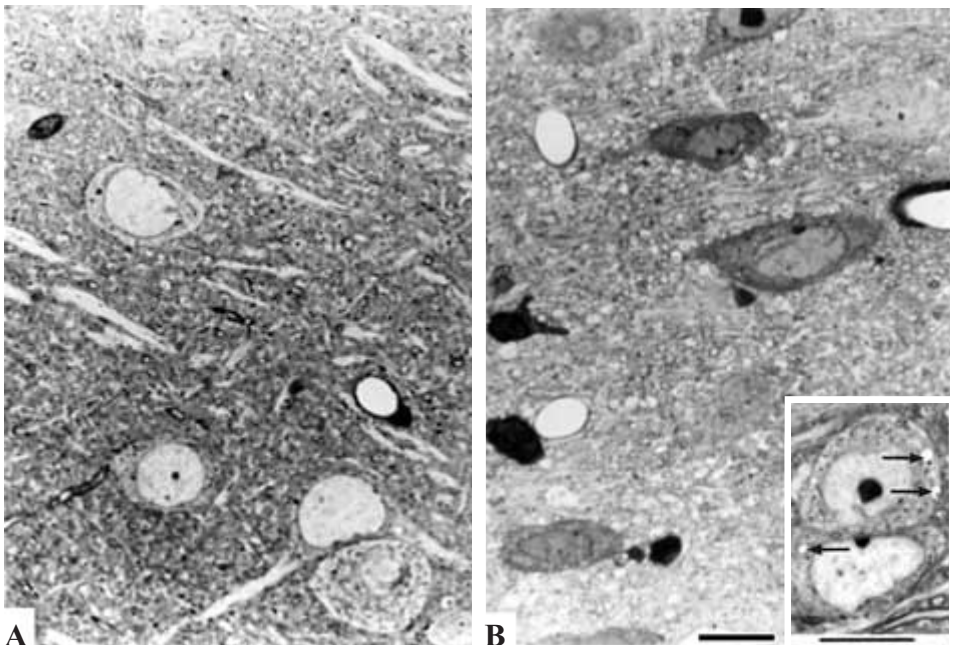

Fig. 7. Semi-thin sections stained with toluidine blue from the cingulate cortex of adult female rats four days after treatment. **A.** After treatment with gacyclidine (20 mg/kg), no evidence of neuronal lesion. The tissue is similar to that of control animals. **B.** After treatment with MK-801 (10 mg/kg), many neurons appear necrotic. In the inset, at higher magnification the typical appearance of a vacuolar neuron (arrows point to vacuoles). Scale bar = 10 μm.

with tremors, sedation, and exophthalmia. With CNS-1102, at all doses tested, the animals exhibited some excitation. At the highest doses (10 and 20 mg/kg) they suffered from severe akinesia 1 h after drug administration. In contrast, animals that received 1 or 5 mg/kg of gacyclidine or its enantiomers behaved similarly to untreated animals. At the highest doses (10 and 20 mg/kg), the animals began to show some signs of excitation. For all doses, the recovery was always better with gacyclidine and its enantiomers than with MK-801 or CNS-1102.

After either 18 h or 4 days, the animals were sacrificed and their brains prepared for examination by electron and light microscopy. As previously described (27), 4 days after treatment with MK-801, light microscopy examination revealed the presence of necrotic neurons (Fig. 7B). Although not reported in previous studies (27,83), vacuolar neurons could also be observed by electron microscopy (Fig. 8B). Indeed, typical images of damaged mitochondria were observed (Fig. 8B, inset). Necrotic neurons were also detected on tissue samples collected 18 h after the treatment (Fig. 9B). After CNS-1102 treatment, necrotic neurons could be observed in the 10 and 20 mg/kg groups. With MK-801 and CNS-1102, higher doses were associated with the detection of more necrotic cells. In addition to suffering neurons, early signs of glial reaction were also detected.

After treatment with gacyclidine, (+)GK11, or (–)GK11, some vacuolar cells could be detected, but only by electron microscopy in the 20-mg/kg group. The observed lesions were small cytoplasmic or intramitochondrial vacuoles (Fig. 8A). In addition, no neuronal

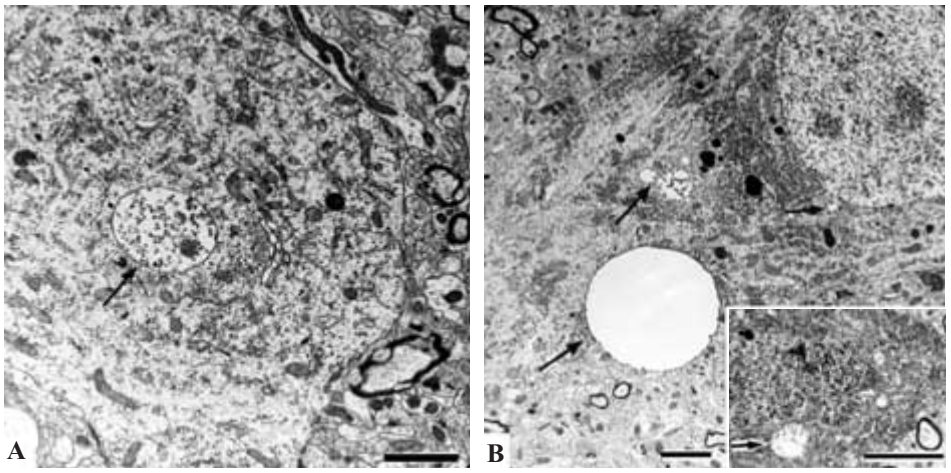

Fig. 8. Appearance of vacuolar profiles in neurons of the cingulate cortex of adult female rats 4 days after treatment (electron microscopy). **A.** Gacyclidine (20 mg/kg). A few neurons display circular vacuoles surrounded by a double membrane containing small vesicles (arrow). **B.** MK-801 (10 mg/kg). Similar vacuoles in large numbers are seen in many neurons (arrows). **Inset**: In addition, dilated mitochondria (arrow) are frequently observed in many neurons. Scale bar = 2 μm.

or glial alterations, such as astrocytic swelling or microglial activation, were seen that could suggest a short-term toxic event had occurred. According to previous observations with other NMDAR antagonists, cytoplasmic vacuoles were often observed in the vicinity of the mitochondria or the Golgi apparatus. Sometimes cleared and slightly dilated mitochondria were observed. In the other groups, both light and electron microscopy were unable to detect any difference from control animals. Even with the 20-mg/kg dose, no necrotic neurons were observed after treatment with gacyclidine or its enantiomers (Figs. 7A and 9A).

Thus, in our study the observations made on tissues of MK-801-treated animals were slightly different from those of previous studies. Indeed, vacuolization has been claimed to occur within 4–6 h following injection and to disappear after 12 h (27). Moreover, necrotic neurons were usually detected from 36–48 h instead of the 18-h interval observed by Sandillon and Privat.

The reasons for these discrepancies are currently unknown. However, considering that MK-801- and gacyclidine-treated animal samples were randomly processed, the results of the neurotoxicity study consistently showed that gacyclidine and its enantiomers display only minimal neurotoxicity in the cingulate cortex compared with the reference compounds. The two time intervals of 18 and 96 h do not allow for complete exclusion of either a very early or a very late neurotoxicity. Indeed, since the animals were not sacrificed and evaluated at 4 h after drug treatment, the initial transient phase of vacuolization observed with MK-801 (27,86) could have been missed for gacyclidine and its enantiomers, and this issue will soon be investigated. Nevertheless, whatever the observations, current evidence from the literature indicates that a short-term toxicity, if it exists, would be totally reversible. Likewise, any long-term toxicity would become evident after 4 days. In this respect, it is worth noting that the minimal alterations encountered in gacyclidine-

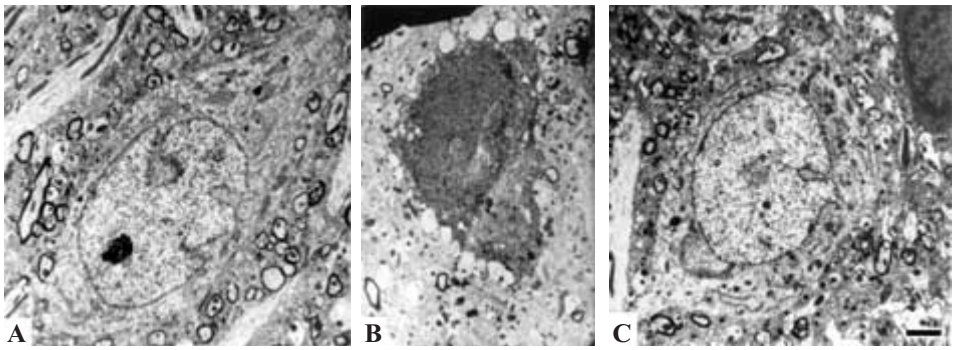

Fig. 9. Characteristic appearance of neuronal perikarya in the cingulate cortex of adult male treated rats sacrificed 18 h after treatment (electron microscopy). **A.** Gacyclidine (20 mg/kg). No evidence of lesion. **B.** MK-801 (10 mg/kg). A typical necrotic neuron surrounded by vacuole profiles. **C.** A neuron from a control animal injected with saline. Scale bar = 1.7 μm.

treated animals (only in the 20 mg/kg group) were found for doses far higher than those yielding major alterations with MK-801 (1 mg/kg, data not shown).

Thus, as a whole, the histological evidence, together with behavioral observations, strongly suggest that gacyclidine and its enantiomers are, at least, far less neurotoxic than MK-801. This cannot be attributed to a lower antagonism at the NMDAR, since both MK-801 and (–)GK11 have an affinity in the nanomolar range for this glutamate receptor (Table 1) (41). Since antagonism of NMDARs is very likely to be responsible for the neurotoxicity of MK-801 and other NMDAR antagonists, the lower neurotoxic potential of gacyclidine and its enantiomers may be explained by the interaction of these compounds with other binding sites. These latter could modulate the blockade of NMDARs and prevent subsequent pathological lesions. The molecular nature of these binding sites needs to be investigated. In any case, the *in vitro* neuroprotective experiments (18) and the results of the neurotoxicity study (Sandillon and Privat, personal communication) indicate that gacyclidine could present a higher benefit/risk ratio for treatment of CNS injuries in humans than other high-affinity NMDAR antagonists that have been tested so far.

NEUROPROTECTION *IN VIVO*

Efficacy in Poisoning by Organophosphorous Agents (49)

Organophosphorous (OP) nerve agents, such as soman, are considered to be potential warfare and terrorism compounds (as has already been seen during the attack using sarin in the Tokyo subway in 1995). They act as irreversible acetylcholinesterase (AChE) inhibitors in the central and peripheral nervous systems. OP intoxication produces hypersalivation, lacrimation, diarrhea, tremor, respiratory distress, convulsions, and seizures. In addition to the cholinergic system, Lallement et al. (50,51) and Sparenborg et al. (104) have established that the excitatory amino acid, glutamate, plays a prominent role in the maintenance of soman-induced seizures and subsequent neuropathology via overstimulation of NMDARs.

The currently recommended treatment for OP poisoning consists of the immediate coinjection of a three-drug cocktail: (1) atropine (a cholinergic antagonist); (2) pralidoxime (a reactivator of OP-inhibited AChE); and (3) diazepam (a benzodiazepine anticonvulsant). The efficacy of this emergency therapy against acute soman poisoning has been tested in Cynomolgus monkeys (55) and has been proven effective against the lethal and epileptic effects of soman. However, despite rapid control of status epilepticus, this treatment neither entirely restores normal electroencephalographic (EEG) activity nor does it totally prevent brain damage (53). The intravenous (i.v.) injection of the three-drug cocktail (atropine, diazepam, and pralidoxime) 30 min after poisoning was unable to rapidly stop seizures, reverse signs of toxicity, and prevent neuronal rarefaction in the frontoparietal cortex when animals exhibited severe signs of poisoning (52).

In contrast, in different protocols of soman poisoning, i.v. injection of gacyclidine in addition to the classical three-drug cocktail has been shown to efficiently improve neurological outcome (52,55). Indeed, gacyclidine was able to stop severe seizures when administered 30 min after poisoning (2 LD_{50}). More importantly, gacyclidine totally prevented soman-induced neuropathology observed in the frontoparietal cortex and the cerebellum of control animals (i.e., injection of atropine, pralidoxime, and diazepam alone). In animals that were not treated with gacyclidine, the neuronal loss observed in the frontoparietal cortex was closely correlated with seizures and is probably linked to the persistence of epileptic activity for 1.5 h after poisoning (52,54). Accordingly, in gacyclidine-treated animals, cortical EEG activity rapidly returned to normal after treatment. Moreover, Purkinje cells are very vulnerable to any form of cerebral hypoxia (14). Their disappearance observed in control primates, which exhibited severe signs of intoxication, could be due to cerebral hypoxia related to the severe and persistent respiratory disorders of these animals. Interestingly, the protection of Purkinje cells afforded by the addition of gacyclidine to the three-drug cocktail could be related to the improvement of brain oxygenation after administration. The fact that gacyclidine may profoundly influence respiration is not surprising since the glutamatergic system plays a pivotal role in central respiratory control (28).

Gacyclidine, therefore, appears to constitute a promising adjuvant therapy to current polymedication to ensure optimal management of severe OP intoxication. However, it should be kept in mind that, after OP poisoning, rapid medical intervention is needed. Indeed, it has been shown that late injection (45 min after intoxication) of the three-drug cocktail failed to stop seizures induced by 2 LD_{50} of soman and to ensure survival or clinical recovery, even when combined with gacyclidine (52). Interestingly, in a military context, when subjects were pretreated with pyridostigmine 1 h before poisoning, a late (+45 min) injection of gacyclidine completely prevented the effects of an 8 LD_{50} soman intoxication (49).

Efficacy on Different Animal Models of Spinal Cord Injury

Gacyclidine neuroprotection and animal models of spinal cord injury (SCI)

One of the problems of pharmacological attenuation of traumatic excitotoxic damage of the spinal cord concerns the apparent acute time course of glutamate release following trauma (22,24,26,28,44,45,79,80). However, significant discrepancies exist between authors with respect to the magnitude of glutamate increase (22,45,67,79). On the one hand, some studies suggest that the post-traumatic rise in extracellular glutamate is fairly short-

lived; on the other hand, clinical studies have reported that glutamate concentrations are significantly increased in the cerebrospinal fluid of brain-injured patients for several days (3,88). Despite these differences and according to the existing evidence, noncompetitive antagonists of NMDARs appear to be an essential factor in the elaboration of a neuroprotective strategy after SCI. It seems that this particular target (i.e., NMDARs) requires a rapid intervention after trauma for maximal beneficial neurological results (31).

Experimental ischemic spinal cord lesion

Studies of the microvasculature after acute SCI have shown that one of the main components of secondary lesion is a disorder of the microcirculation. As described by Watson et al. (115,116), an experimental photochemical lesion optimally reproduces the conditions of microvascular injury involved in deterioration due to SCI. It is a reproducible technique and does not require major surgical manipulation (laminectomy). However, despite the neurological consequences of this injury, it nevertheless constitutes a vascular lesion and cannot be considered to represent a traumatic lesion. In fact, the photochemical method of injury by dorsal irradiation in the rat differs from the usual mechanisms of lesions in human SCI. However, 30% post-irradiation tissue necrosis is sufficient to induce paraplegia.

The efficacy of gacyclidine was analyzed on a model of ischemic SCI in order to determine the optimal dose and time-window for treatment. Timing and doses of gacyclidine were defined in accordance with previous studies using TCP on experimental cerebral ischemia in the rat (23) and *in vitro* experimentation (95). Photochemical lesion was carried out using the Watson ischemic photochemical model (31,94,116). Gacyclidine was administered i.v., on the one hand, at different doses 10 min after SCI (1, 2.5, and 5 mg/kg), and on the other hand, at a dose of 1 mg/kg at different times (10, 30, 60, and 120 min after SCI). Afterwards, the functional, electrophysiological, and histopathological behavior of the studied populations were analyzed until stabilization of the cord lesion, which occurred around the third week (9,29,89,99,107).

Gacyclidine was able to reduce the effects of secondary injury following an ischemic lesion of the spinal cord, which was reflected by the measured parameters in treated animals compared with untreated animals. Moreover, one of the major effects of gacyclidine was to limit the extent of spinal cord damage at and above the level of injury. The best dose-effect response was observed with 1 mg/kg of gacyclidine, which was in accordance with previous papers (23,95), and the best time-effect response was observed when treatment was administered early after SCI (10 and 30 min).

The neuroprotected groups showed significant recovery of motor performances compared with the untreated group. Motor performances corresponded mostly with defined walking patterns with only a very slight deficit; the performances of the injured untreated group corresponded with frequent and vigorous movements of the hind limbs with no weight-bearing or defined walking pattern. The motor outcome resulting from CNS drive reflects several interdependent mechanisms, which are all more or less directly related to the extent of the lesion (80,89,94). This postulate was confirmed by anatomical and histological examination of spinal cord sections. They showed a larger undamaged area at the level of the lesion in the treated groups with significant limitation of the extent of the lesion at this level when a 1-mg/kg dose of gacyclidine was administered. The functional outcome was not strictly correlated with histological examination of the area of damage at the level of the injury, but the undamaged cross-sectional area was significantly greater in

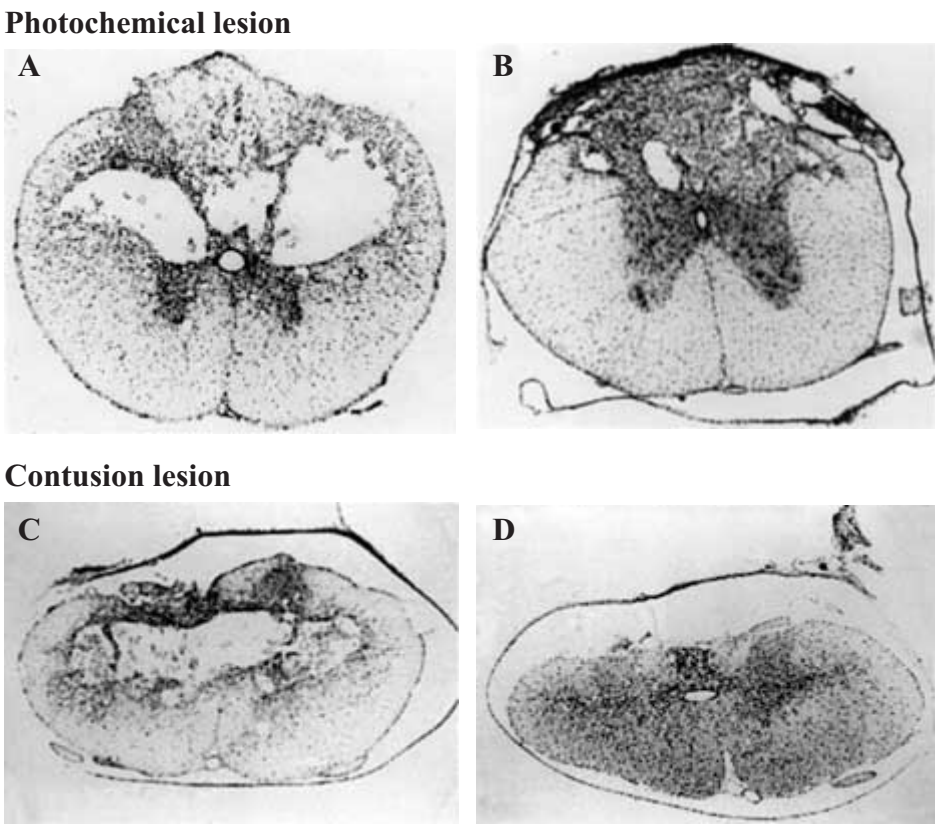

Fig. 10. Cryostat cross sections of the spinal cord at the epicenter of the lesion. **A.** Photochemical lesion in an untreated animal. **B.** Photochemical lesion in a 1 mg/kg gacyclidine-treated (10 min after lesioning) animal. **C.** Contusive lesion in an untreated animal**. D.** contusive lesion in a 1 mg/kg gacyclidine-treated (10 min after lesioning) animal. See the reduction of the lesioned area in gacyclidine-treated animals. Nissl staining, ×22.

treated groups (63 ± 4.3 to 75 ± 6.5% vs. 20.8 ± 1.9%, Fig. 10A, B). One of the major findings was the reduction of histological damage above the level of the injury (5.6 mm from the epicenter) in treated groups, which reflects, according to the anatomical characteristics of the Sprague–Dawley laboratory rat, the preservation of one metameric level (37) and, consequently, the reduction of the functional deficit. Extension of histological damage was generally observed only in untreated animals. The mechanisms of extension of the lesion are probably related to local vascular changes following acute trauma. Gacyclidine appeared to limit the failure of autoregulation mechanisms, including loss of microcirculation, probably due to local vasospasm. Finally, electrophysiological data concerning somatosensory-evoked potential amplitudes were in accordance with previous findings, suggesting that local circuits may have been partially spared at the epicenter of the lesion, but also at a distance from the epicenter, via intersegmental circuitry (73).

Experimental contusive spinal cord lesion

Weight-drop. While treatment with gacyclidine was effective in a model of ischemic SCI when given after lesioning (31), another question, whether treatment with gacyclidine

could be effective when given after a contusive experimental spinal cord lesion where damage involves direct (mechanical disruption of neural pathways) and indirect (secondary or delayed) mechanisms, remained unanswered. The neuroprotective time-dependent effects of gacyclidine on a contusive model of SCI in the rat were analyzed, completing the neuroprotective dose- and time-dependent studies of gacyclidine on the ischemic experimental model (31). The neuroprotective efficacy of gacyclidine on functional outcome after experimental contusive spinal cord lesion in rats was determined relative to the time of administration of the drug after SCI (10, 30, 60, and 120 min). The single dose required in the ischemic model of SCI to induce the most significant inhibition of spinal cord damage was determined to be 1 mg/kg; repeated doses did not provide any additional neurological improvement (Feldblum, unpublished observations).

The contusive experimental model of SCI offers the advantage of being clinically pertinent, since the majority of human cord traumas involve damage caused by rapid movements of the vertebral column (acceleration-deceleration) and impact of fractured bone against the spinal cord (1). This model (1) was refined for use in rats by Wrathall et al. (117) and shown to provide a reliable and reproducible injury (7); some studies have established that the histopathological features of this model are similar to those previously reported in larger species (8,80). The spinal cord was injured using a variation of the Wrathall contusion model by dropping a calibrated weight down from a specified height, which provoked a direct impact over the surgically exposed (T8 laminectomy) medullar T8–T9 segment (1,47,117). Subsequently, the functional, electrophysiological, and histopathological behaviors of the studied populations were analyzed until the stabilization of the cord lesion around the third week (30).

It was noted that gacyclidine was also able to reduce the effects of secondary injury following experimental contusive lesion of the spinal cord (reduction reflected by the measured variables in treated animals compared with untreated animals) and that the best response was observed 10 min after SCI.

In general, animals receiving a contusive weight-drop injury showed profound neurological impairment immediately after injury, followed by a variable degree of functional recovery until the third week. Neuroprotected animals showed a higher mean motor recovery. Motor performance corresponded to defined walking patterns with only a very slight deficit. In contrast, frequent and vigorous movements of the hind limbs with no weight-bearing or defined walking pattern were found in the injured untreated group. Functional behavior was in accordance with histopathological findings at the epicenter of the lesion, which showed a significant undamaged area in treated animals (30.1 ± 3.2 to $41.5 \pm 4.4\%$ vs. $17.2 \pm 2.7\%$ in the untreated group, Fig. 10C, D). With regard to the extension of the lesion rostrally to the epicenter, as described above, a greater damaged area was noted in untreated rats. Finally, somesthetic-evoked potential amplitudes were found to be slightly greater in the treated groups than in the untreated group, which also suggested a partially spared local medullar circuitry (73).

It was shown that complex coordinated motor functions, quantified by motor performances on the open field-walking test, provided a good prediction of lesion histopathology (47). Thus, increased functional ability, as measured by open field walking and inclined-plane stability tests, was associated with lesser damage in the total cord area. Moreover, electrophysiological data correlated well with the severity of the injury and,

consequently, indirectly evaluated motor pathway damage as described by Raines et al. in 1988 (96).

Subdural inflatable balloon. As mentioned before, the contusive experimental model of SCI offers the advantage of being clinically pertinent, as it shares homologies with human injury since it is a closed contusion with similar histopathological changes characterized by the formation of a cystic cavity. Based on a method initially described by Tarlov in 1954 (107) and adapted to the rat by Martin et al. (63), a contusive SCI was induced by introducing subdurally an inflatable balloon, rostrally to a T9–T10 laminectomy (25). Varying the degree and the duration of compression permitted a reversible paraplegic state.

In the study conducted by Feldblum et al. (26), gacyclidine was administered 10 min after spinal cord compression at different doses (0.03, 0.1, 0.3, 1, and 3 mg/kg) as a single injection. In addition, a prolonged delivery of 1 mg/kg per day for 7 days was used (unpublished observations). After the lesion, functional and histopathological behaviors were measured over a period of 3 weeks after SCI. This study allowed for quantification of the neuroprotection by studying the kinetics of recovery and the efficacy of the treatment to accelerate the recovery process. Moreover, characterization of the histopathological changes completed the quantification of cellular neuroprotection.

Gacyclidine was not able to prevent occurrence of paraplegia after the contusion, but it significantly accelerated the recovery of locomotion so that treated animals recovered twice as fast. The authors found that high and low doses of gacyclidine gave similar neuroprotection, which suggests an inverse U-shaped drug effect, such as that observed by Smith et al. (103). Nevertheless, the most significant neuroprotection was induced by 1 mg/kg of gacyclidine while the recovery process was slowed down in the group receiving 3 mg/kg, which suggests that adverse effects are present at this dose. Prolonged treatment for 1 week did not provide any additional improvement in recovery of locomotion.

Histopathology demonstrated cellular neuroprotection with only a single dose, suggesting that gacyclidine overdose is not beneficial to the tissue. It was concluded that, if administered at high concentrations or during a prolonged period, gacyclidine induces adverse effects. Despite locomotor recovery, the model of inflatable balloon is associated with interruption of the major long spinal tract (dorsal funiculi, pyramidal tracts) at the epicenter of the injury. In the early stages, extensive hemorrhagic necrosis is observed. During the subacute period, the site of the lesion is occupied by numerous macrophages. Finally, at the chronic stage, central hemorrhagic necrosis is replaced by a large cystic cavity and a vascular connective tissue scar, and astrocytic gliosis becomes visible. The development of a glial scar, characterized by astrocyte proliferation and hypertrophy in response to trauma, has been extensively documented (70,76,98). It has been considered to participate in the physical and chemical barrier preventing axonal regeneration. In contrast to the observations of Smith et al. (103, see below), Feldblum et al. (26) demonstrated that gacyclidine was able to induce cellular neuroprotection since both the cystic cavity and the astrogliosis were reduced. Feldblum's data suggest a good correlation between behavioral neuroprotection and cellular neuroprotection, and they indicate that a single dose of gacyclidine, administered 10 min after SCI, constitutes a promising approach to limit early cytotoxic damage, promote motor recovery, and limit cellular damage induced by spinal cord compression.

Efficacy on One Model of Traumatic Brain Injury (TBI)

Gacyclidine neuroprotection and animal models of TBI

In the study conducted by Smith et al. (103), the effectiveness of gacyclidine in reducing the behavioral and histopathological consequences of experimentally induced TBI in adult rats was evaluated. The model of TBI consisted of bilateral contusion of the medial frontal cortex, which is a highly effective and replicable model to study the behavioral, anatomical, and physiological changes that occur after TBI, and which simulates the type of head trauma often seen in humans (64). The behavioral deficits caused by this type of brain injury are severe and long lasting (42). In the work of Smith et al. (103), the contusion was carried out using a calibrated impactor as described previously by Hoffman et al. (42). In this model, the loss of neurons expressing NMDARs was associated with impaired learning and memory following TBI. Bilateral damage to the medial frontal cortex in rats induced persistent deficits in cognitive and sensorimotor performances. Different doses of gacyclidine were tested 10 min after TBI (0.3, 0.1, and 0.03 mg/kg). Behavioral and histopathological data were measured over a period of 3 weeks after TBI.

Animals treated with 0.1 mg/kg of gacyclidine performed significantly better than those treated with 0.01 and 0.3 mg/kg, which possibly indicates the drug has a U-shaped or sigmoidal effect. Despite the observation of enhanced behavioral recovery, none of the treated animals reached levels of performance equivalent to those of the intact controls. Animals given 0.1 mg/kg of gacyclidine had more surviving magnocellular neurons in the nucleus basalis and more surviving neurons in the medial dorsal nucleus of the thalamus than untreated animals. Gacyclidine apparently increased the survival of neurons in remote subcortical areas of the brain that usually degenerate following medial frontal cortex injury. These effects may be due to the capacity of gacyclidine to prevent glutamate toxicity, a finding that is consistent with the literature for compounds acting on similar receptors and/or having similar mechanisms of action (44,66,102).

Thus, the authors pointed out that gacyclidine improved behavioral performances after TBI and enhanced survival of neurons in the medial dorsal nucleus of the thalamus and magnocellular neurons in the nucleus basalis magnocellularis. This wide range of effects was interpreted to be an indication that gacyclidine may have multiple sites of action in the CNS apart from its NMDAR site. According to the authors, the effect of gacyclidine after systemic administration in a complex pathology like TBI may be also more general and affect physiological functions outside of the CNS. NMDAR antagonists have been shown in general to have effects on the cardiovascular system (61,77), the body's water balance (20,48), and secretion and release of hormones (21,93), as well as on the respiratory tract and oxygen consumption (6,59). Thus, the observed functional recovery after treatment with gacyclidine could be due, at least partially, to the systemic effects mentioned earlier.

CONCLUSIONS

Secondary injury after a CNS trauma involves complex neurochemical, cellular, and molecular cascades leading to a variable degree of neurological impairment (33,65,66). It

is clear that critical periods following injury exist during which specific pharmacological interventions must be initiated in the hope that treatments will be maximally effective. Based on the available evidence, noncompetitive NMDAR antagonists, such as gacyclidine, appear to be an essential component in the conception of neuroprotective strategies.

Taken together, the pharmacological data presented here indicate that the neuroprotection afforded by gacyclidine is likely to be related to its ability to antagonize glutamate effects at the NMDAR. This aspect of gacyclidine's mechanism of action is similar to that of other NMDAR antagonists. Its specificity lies in its lack, or low levels, of direct neurotoxicity that appears at doses that are far higher than those used for neuroprotection and those found with MK-801. This difference between gacyclidine and MK-801 cannot be explained by different interactions at the NMDAR. Their affinities are identical and they have the same potency of inhibition of glutamate-induced cell death. Gacyclidine is lipophilic, penetrates the CNS easily (78), and is as potent as MK-801 *in vivo*. Therefore, the interaction of gacyclidine with non-NMDA binding sites is likely to be implicated in its neuroprotective efficacy. Non-NMDA binding sites are present throughout the CNS but it is not yet established whether they constitute a homogenous population (40). They are abundant in the cerebellum, particularly in the molecular layer where they are probably located on the dendritic tree of Purkinje cells (39). Autoradiographic and biochemical studies will help to further characterize non-NMDA binding sites and to determine their contribution in neuroprotection. These sites are likely to play a key role in the effects of gacyclidine. Indeed, gacyclidine is currently under clinical evaluation for brain and spinal cord traumatic injury and, in contrast to most studies involving NMDAR antagonists (56), no adverse effects have prevented the course of the trial (15,106).

Other biological strategies for postlesional intervention, which are expected to result in restoration of function (i.e., through axonal regeneration and glial scar control) need an optimal environment to be effective and could benefit from early antagonism of NMDARs. Significant differences exist in the extensive literature published to date with respect to the behavior of numerous endogenous neurochemical systems after spinal cord or brain injuries. Excitatory amino acids, acetylcholine, endogenous opioids, catecholamines, serotonin, cytokines, free radicals, platelet activating factor, steroids, magnesium, and ion channels are some of the physiological compounds participating in the pathophysiology of secondary injury (65,66,108). Unfortunately and despite many promising results from potentially neuroprotective pharmacological agents, detailed kinetics of the evolution of the physiological substances involved in the pathophysiology of posttrauma are still lacking. Future progress toward the proposal of a global strategy in SCI and TBI requires a systematic study of the time course of anatomical, biochemical, and physiological events that occur. This information will permit us to define an algorithm, which may lead to an optimal sequentially timed combination of rational therapeutic strategies.

Glutamate is at an early stage of its development and NMDA strategy appears to be a part of general antiischemic strategy. Because of its relatively safe profile, gacyclidine could be valuable as a part of a global therapeutic scheme.

Acknowledgments. The authors would like to thank the Beaufour-Ipsen Company for providing the content of internal reports. Thanks are also due to Drs. A. Privat and J. M. Kamenka for their suggestions during the preparation of the manuscript. Special thanks are due to J. R. Teilhac for the preparation of the figures. The authors also thank S. De La Rue for the careful correction of the manuscript.

REFERENCES

1. Allen AR. Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column. A preliminary study. *JAMA* 1911;57:878–880.
2. Allen HL, Iversen LL. Phencyclidine, dizocilpine, and cerebrocortical neurons. *Science* 1990;247:221.
3. Baker AJ, Moulton RJ, MacMillan VH, Shedden PM. Excitatory amino acids in cerebrospinal fluid following traumatic brain injury in humans. *J Neurosurg* 1993;79:369–372.
4. Baudy RB. Agents for the treatment of neurodegenerative diseases: July–December 1997. *Exp Opin Ther Patents* 1998;8:395–438.
5. Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem* 1984;43:1369–1374.
6. Bissonnette JM, Hohimer AR, Knopp SJ. Non-NMDA receptors modulate respiratory drive in fetal sheep. *J Physiol (Lond)* 1997;501:415–423.
7. Black P, Markowitz RS, Damjanov I, et al. Models of spinal cord injury. Part 3. Dynamic load technique. *Neurosurgery* 1988;22:51–60.
8. Bresnahan JC, King JS, Martin GF, Yashon D. A neuroanatomical analysis of spinal cord injury in the rhesus monkey (*Macaca mulatta*). *J Neurol Sci* 1976;28:521–542.
9. Cameron T, Prado R, Watson BD, Gonzalez-Carvajal M, Holets VR. Photochemically induced cystic lesion in the rat spinal cord. I. Behavioral and morphological analysis. *Exp Neurol* 1990;109:214–223.
10. Cazaux JB, Dafniet M, Kamenka JM, Manginot E. Dérivés du thiénylcyclohexane, des procédés pour leur préparations et leur utilisation en tant que produit cyclohéxylés. FR27516 5L, 1998.
11. Chaudieu I, Vignon J, Chicheportiche M, Kamenka JM, Trouiller G, Chicheportiche R. Role of the aromatic group in the inhibition of phencyclidine binding and dopamine uptake by PCP analogs. *Pharmacol Biochem Behav* 1989;32:699–705.
12. Choi DW, Koh JY, Peters S. Pharmacology of glutamate neurotoxicity in cortical cell culture: Attenuation by NMDA antagonists. *J Neurosci* 1988;8:185–196.
13. Conley EC. N-Methyl-D-Aspartate (NMDA) selective glutamate receptor channels. In: Conley EC, ed. *The Ion Channel*, *Facts Book*. London: Acad. Press, 1996.
14. Corsellis J, Meldrum B. Epilepsy. In: Blackwood W, Corsellis J, eds. *Greenfield's Neuropathology*. London: Edward Arnold, 1976:771–795.
15. D'Arbigny P, Tadié M, Vigue B, Mathé JF, Chabrier PE, Carli P. Protec: A dose effect study of gacyclidine in patients with severe brain injury. *Soc Neurosci Abstr* 1999;25(Part 1):1090.
16. Doble A. Excitatory amino acid receptors and neurodegeneration. *Therapie* 1995;50:319–337.
17. Drian MJ, Kamenka JM, Pirat JL, Privat A. Non-competitive antagonists of N-methyl-D-aspartate prevent spontaneous neuronal death in primary cultures of embryonic rat cortex. *J Neurosci Res* 1991;29:133–138.
18. Drian MJ, Kamenka JM, Privat A. *In vitro* neuroprotection against glutamate toxicity provided by novel non-competitive N-methyl-D-aspartate antagonists. *J Neurosci Res* 1999;57:927–934.
19. Ellison G. Competitive and non-competitive NMDA antagonists induce similar limbic degeneration. *Neuroreport* 1994;5:2688–2692.
20. Espanol MT, Xu Y, Litt L, et al. Modulation of edema by dizocilpine, kynurenate, and NBQX in respiring brain slices after exposure to glutamate. *Acta Neurochir Suppl (Wien)* 1994;60:58–61.
21. Estienne MJ, Harter-Dennis JM, Barb CR, Hartsock TG, Campbell RM, Armstrong JD. N-methyl-D,L-aspartate-induced growth hormone secretion in barrows: Possible mechanisms of action. *J Anim Sci* 1996;74: 597–602.
22. Faden AI, Demediuk P, Panter SS, Vink R. The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science* 1989;244:798–800.
23. Faden AI, Simon RP. A potential role for excitotoxins in the pathophysiology of spinal cord injury. *Ann Neurol* 1988;23:623–626.
24. Farooque M, Hillered L, Holtz A, Olsson Y. Changes of extracellular levels of amino acids after graded compression trauma to the spinal cord: An experimental study in the rat using microdialysis. *J Neurotrauma* 1996;13:537–548.
25. Feldblum S. Method for screening factors on a paralysis model and method for obtaining this model. US Patent 5724995, 1994.
26. Feldblum S, Arnaud S, Simon M, Rabin O, D'Arbigny P. Efficacy of a new neuroprotective agent, gacyclidine, in a model of rat spinal cord injury. *J Neurotrauma* 2000;17:1079–1093.

27. Fix AS, Horn JW, Wightman KA, et al. Neuronal vacuolization and necrosis induced by the noncompetitive 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.
28. Foutz AS, Champagnat J, Denavit-Saubie M. Involvement of N-methyl-D-aspartate (NMDA) receptors in respiratory rhythmogenesis. *Brain Res* 1989;500:199–208.
29. Gale K, Kerasidis H, Wrathall JR. Spinal cord contusion in the rat: Behavioral analysis of functional neurologic impairment. *Exp Neurol* 1985;88:123–134.
30. Gaviria M, Privat A, d'Arbigny P, Kamenka J, Haton H, Ohanna F. Neuroprotective effects of a novel NMDA antagonist, gacyclidine, after experimental contusive spinal cord injury in adult rats. *Brain Res* 2000;874:200–209.
31. Gaviria M, Privat A, d'Arbigny P, Kamenka JM, Haton H, Ohanna F. Neuroprotective effects of gacyclidine after experimental photochemical spinal cord lesion in adult rats: Dose-window and time-window effects. *J Neurotrauma* 2000;17:19–30.
32. Geneste P, Kamenka JM, Ung SN, Herrmann P, Goudal R, Trouiller G. Détermination conformationnelle de dérivés de la phencyclidine en vue d'une corrélation structure-activité. *Eur J Med Chem* 1979;14:301–308.
33. Gimenez y Ribotta M, Privat A. Biological interventions for spinal cord injury. *Curr Opin Neurol* 1998;11: 647–654.
34. Greenamyre JT, Young AB, Penney JB. Quantitative autoradiography of L-[^{3}H]glutamate binding to rat brain. *Neurosci Lett* 1983;37:155–160.
35. Halpain S, Wieczorek CM, Rainbow TC. Localization of L-glutamate receptors in rat brain by quantitative autoradiography. *J Neurosci* 1984;4:2247–2258.
36. Hamon J, Espaze F, Vignon J, Kamenka JM. The search for TCP analogs binding to the low affinity PCP receptor sites in the rat cerebellum. *Eur J Med Chem* 1999;34:125–135.
37. Hebel R, Stromberg MW. *Anatomy and embryology of the laboratory rat*. Wörthsee: Biomed. Verlag, 1986.
38. Hirbec H, Kamenka JM, Privat A, Vignon J. Binding properties of [^{3}H]gacyclidine in the rat central nervous system. *Eur J Pharmacol* 2000;388:235–239.
39. Hirbec H, Kamenka JM, Privat A, Vignon J. Characterisation of "non-NMDA" binding sites for gacyclidine in the rat cerebellar and telencephalic structures. *J Neurochem* 2001;77:190–201.
40. Hirbec H, Kamenka JM, Privat A, Vignon J. Interaction of [^{3}H]gacyclidine enantiomers with "non-NMDA" receptors in the rat central nervous system. *Brain Res* 2001;894:189–192.
41. Hirbec H, Teilhac JR, Kamenka JM, Privat A, Vignon J. Binding properties of [^{3}H]gacyclidine (cis(pip/me)-1-[1-(2-thienyl)-2-methylcyclohexylpiperidine] enantiomers in the rat central nervous system. *Brain Res* 2000;859:177–192.
42. Hoffman SW, Fulop Z, Stein DG. Bilateral frontal cortical contusion in rats: Behavioral and anatomic consequences. *J Neurotrauma* 1994;11:417–431.
43. Hollmann M, Heinemann S. Cloned glutamate receptors. *Annu Rev Neurosci* 1994;17:31–108.
44. Jenkins LW, Lyeth BG, Lewelt W, et al. Combined pretrauma scopolamine and phencyclidine attenuate posttraumatic increased sensitivity to delayed secondary ischemia. *J Neurotrauma* 1988;5:275–287.
45. Katayama Y, Becker DP, Tamura T, Hovda DA. Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. *J Neurosurg* 1990;73:889–900.
46. Kito S, Miyoshi R, Nomoto T. Influence of age on NMDA receptor complex in rat brain studied by *in vitro* autoradiography. *J Histochem Cytochem* 1990;38:1725–1731.
47. Kuhn PL, Wrathall JR. A mouse model of graded contusive spinal cord injury. *J Neurotrauma* 1998;15: 125–140.
48. Kuroda Y, Fujisawa H, Strebel S, Graham DI, Bullock R. Effect of neuroprotective N-methyl-D-aspartate antagonists on increased intracranial pressure: Studies in the rat acute subdural hematoma model. *Neurosurgery* 1994;35:106–112.
49. Lallement G, Baubichon D, Clarencon D, Galonnier M, Peoc'h M, Carpentier P. Review of the value of gacyclidine (GK-11) as adjuvant medication to conventional treatments of organophosphate poisoning: Primate experiments mimicking various scenarios of military or terrorist attack by soman. *Neurotoxicology* 1999;20:675–684.
50. Lallement G, Carpentier P, Collet A, Pernot-Marino I, Baubichon D, Blanchet G. Effects of soman-induced seizures on different extracellular amino acid levels and on glutamate uptake in rat hippocampus. *Brain Res* 1991;563:234–240.
51. Lallement G, Carpentier P, Pernot-Marino I, Baubichon D, Collet A, Blanchet G. Involvement of the different rat hippocampal glutamatergic receptors in development of seizures induced by soman: An autoradiographic study. *Neurotoxicology* 1991;12:655–664.

52. Lallement G, Clarencon D, Galonnier M, Baubichon D, Burckhart MF, Peoc'h M. Acute soman poisoning in primates neither pretreated nor receiving immediate therapy: Value of gacyclidine (GK-11) in delayed medical support. *Arch Toxicol* 1999;73:115–122.
53. Lallement G, Clarencon D, Masqueliez C, et al. Nerve agent poisoning in primates: Antilethal, anti-epileptic and neuroprotective effects of GK-11. *Arch Toxicol* 1998;72:84–92.
54. Lallement G, Delamanche IS, Pernot-Marino I, et al. Neuroprotective activity of glutamate receptor antagonists against soman-induced hippocampal damage: Quantification with an omega 3 site ligand. *Brain Res* 1993;618:227–237.
55. Lallement G, Mestries JC, Privat A, et al. GK 11: Promising additional neuroprotective therapy for organophosphate poisoning. *Neurotoxicology* 1997;18:851–856.
56. Lee JM, Zipfel GJ, Choi DW. The changing landscape of ischaemic brain injury mechanisms. *Nature* 1999; 399:A7–A14.
57. Levallois C, Calvet MC, Petite D, Kamenka JM. Neuroprotective effect of N-methyl-D-aspartate non-competitive antagonists (arylcyclohexylamine derivatives) on human cultured spinal cord cells. *Int J Dev Neurosci* 1994;12:547–555.
58. Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med* 1994;330:613–622.
59. Lu X, Sinha AK, Weiss HR. Effects of excitatory amino acids on cerebral oxygen consumption and blood flow in rat. *Neurochem Res* 1997;22:705–711.
60. Lucas D, Newhouse J. The toxic effect of sodium-Lglutamate on inner layers of retina. *AMA Arch Ophtalmol* 1957;58:193–201.
61. Maione S, Pallotta M, Leyva J, Palazzo E, Rossi F. Effects of diethylenetriamine on NMDA-induced increase of blood pressure in rats. *Pharmacol Biochem Behav* 1998;59:233–237.
62. Maragos WF, Penney JB, Young AB. Anatomic correlation of NMDA and ^{3}H-TCP-labeled receptors in rat brain. *J Neurosci* 1988;8:493–501.
63. Martin D, Schoenen J, Delree P, et al. Experimental acute traumatic injury of the adult rat spinal cord by a subdural inflatable balloon: Methodology, behavioral analysis, and histopathology. *J Neurosci Res* 1992;32: 539–550.
64. Mattson AJ, Levin HS. Frontal lobe dysfunction following closed head injury. A review of the literature. *J Nerv Ment Dis* 1990;178:282–291.
65. McIntosh TK. Novel pharmacologic therapies in the treatment of experimental traumatic brain injury: A review. *J Neurotrauma* 1993;10:215–261.
66. McIntosh TK, Juhler M, Wieloch T. Novel pharmacologic strategies in the treatment of experimental traumatic brain injury: 1998. *J Neurotrauma* 1998;15:731–769.
67. McIntosh TK, Vink R, Soares H, Hayes R, Simon R. Effect of noncompetitive blockade of N-methyl-D-aspartate receptors on the neurochemical sequelae of experimental brain injury. *J Neurochem* 1990;55: 1170–1179.
68. Meldrum BS. Excitatory amino acid receptors and disease. *Curr Opin Neurol Neurosurg* 1992;5:508–513.
69. Michaud M, Warren H, Drian MJ, et al. Homochiral structures derived from 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP) are non-competitive antagonists of glutamate at NMDA receptor sites. *Eur J Med Chem* 1994;29:869–876.
70. Miller RH, Abney ER, David S, et al. Is reactive gliosis a property of a distinct subpopulation of astrocytes? *J Neurosci* 1986;6:22–29.
71. Mishina M, Mori H, Araki K, et al. Molecular and functional diversity of the NMDA receptor channel. *Ann NY Acad Sci* 1993;707:136–152.
72. Mody I, MacDonald JF. NMDA receptor-dependent excitotoxicity: The role of intracellular Ca^{2+} release. *Trends Pharmacol Sci* 1995;16:356–359.
73. Molander C, Xu Q, Grant G. The cytoarchitectonic organization of the spinal cord in the rat. I. The lower thoracic and lumbosacral cord. *J Comp Neurol* 1984;230:133–141.
74. Monaghan DT, Cotman CW. Distribution of N-methyl-D-aspartate-sensitive L-[^{3}H]glutamate-binding sites in rat brain. *J Neurosci* 1985;5:2909–2919.
75. Mori H, Mishina M. Structure and function of the NMDA receptor channel. *Neuropharmacology* 1995;34:1219–1237.
76. Morin-Richaud C, Feldblum S, Privat A. Astrocytes and oligodendrocytes reactions after a total section of the rat spinal cord. *Brain Res* 1998;783:85–101.
77. Muir KW, Lees KR. Clinical experience with excitatory amino acid antagonist drugs. Stroke 1995;26: 503–513.
78. Muzet M, D'Arbigny P, Luthringer R, et al. Effects of gacyclidine, a noncompetitive NMDA receptor antagonist, in human healthy volunteers. *Soc Neurosci Abstr* 1999;1(Part 1):1090.

79. Nilsson P, Hillered L, Ponten U, Ungerstedt U. Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. *J Cereb Blood Flow Metab* 1990;10: 631–637.
80. Noble LJ, Wrathall JR. Spinal cord contusion in the rat: Morphometric analyses of alterations in the spinal cord. *Exp Neurol* 1985;88:135–149.
81. Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* 1969;164:719–721.
82. Olney JW. Excitotoxic amino acids and neuropsychiatric disorders. *Annu Rev Pharmacol Toxicol* 1990;30:47–71.
83. Olney JW. Neurotoxicity of NMDA receptor antagonists: An overview. *Psychopharmacol Bull* 1994;30: 533–540.
84. Olney JW, Ho OL, Rhee V. Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. *Exp Brain Res* 1971;14:61–76.
85. Olney JW, Labruyere J, Price MT. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science* 1989;244:1360–1362.
86. Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA. NMDA antagonist neurotoxicity: Mechanism and prevention. *Science* 1991;254:1515–1518.
87. Ozawa S. Permeation of calcium through glutamate receptor channels. *Semin Neurosci* 1996;8:261–269.
88. Palmer AM, Marion DW, Botscheller ML, Bowen DM, DeKosky ST. Increased transmitter amino acid concentration in human ventricular CSF after brain trauma. *Neuroreport* 1994;6:153–156.
89. Pencalet P, Ohanna F, Poulat P, Kamenka JM, Privat A. Thienylphencyclidine protection for the spinal cord of adult rats against extension of lesions secondary to a photochemical injury. *J Neurosurg* 1993;78: 603–609.
90. Perl TM, Bedard L, Kosatsky T, Hockin JC, Todd EC, Remis RS. An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *N Engl J Med* 1990;322:1775–1780.
91. Petralia RS, Wang YX, Wenthold RJ. The NMDA receptor subunits NR2A and NR2B show histological and ultrastructural localization patterns similar to those of NR1. *J Neurosci* 1994;14:6102–6120.
92. Petralia RS, Yokotani N, Wenthold RJ. Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. *J Neurosci* 1994;14: 667–696.
93. Pinilla L, Tena-Sempere M, Gonzalez D, Aguilar E. Positive role of non-N-methyl-D-aspartate receptors in the control of growth hormone secretion in male rats. *J Endocrinol Invest* 1996;19:353–358.
94. Prado R, Dietrich WD, Watson BD, Ginsberg MD, Green BA. Photochemically induced graded spinal cord infarction. Behavioral, electrophysiological, and morphological correlates. *J Neurosurg* 1987;67:745–753.
95. Privat A, Drian MJ, Pirat JL, Rondouin G, Kamenka JM. Protection neurale par les phencyclidines: Étude sur des modèles *in vitro*. *Actual Chim Ther* 1990;17:187–200.
96. Raines A, Dretchen KL, Marx K, Wrathall JR. Spinal cord contusion in the rat: Somatosensory evoked potentials as a function of graded injury. *J Neurotrauma* 1988;5:151–160.
97. Regan RF, Choi DW. Glutamate neurotoxicity in spinal cord cell culture. *Neuroscience* 1991;43:585–591.
98. Reier PJ, Stensas LJ, Guth L. The astrocytic scar has an impediment to regeneration in the central nervous system. In: Kao CC, Bunge RP, Reier PJ, eds. *Spinal Cord Reconstruction*. New York: Raven Press, 1983:163–196.
99. Rivlin AS, Tator CH. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. *J Neurosurg* 1977;47:577–581.
100. Rondouin G, Drian MJ, Chicheportiche R, Kamenka JM, Privat A. Non-competitive antagonists of N-methyl-D-aspartate receptors protect cortical and hippocampal cell cultures against glutamate neurotoxicity. *Neurosci Lett* 1988;91:199–203.
101. Scatton B. Excitatory amino acid receptor antagonists: A novel treatment for ischemic cerebrovascular diseases. *Life Sci* 1994;55:2115–2124.
102. Smith DH, Okiyama K, Gennarelli TA, McIntosh TK. Magnesium and ketamine attenuate cognitive dysfunction following experimental brain injury. *Neurosci Lett* 1993;157:211–214.
103. Smith JS, Fulop L, Levinsohn SA, Darrell RS, Stein G. Effects of the novel NMDA antagonist gacyclidine on recovery from medial frontal cortex contusion injury in rats. *Neural Plast* 2000;7:73–91.
104. Sparenborg S, Brennecke LH, Jaax NK, Braitman DJ. Dizocilpine (MK-801) arrests status epilepticus and prevents brain damage induced by soman. *Neuropharmacology* 1992;31:357–368.
105. Subramaniam S, McGonigle P. Quantitative autoradiographic characterization of the binding of (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine ([^{3}H]MK-801) in rat brain: Regional effects of polyamines. *J Pharmacol Exp Ther* 1991;256:811–819.

106. Tadie M, d'Arbigny P, Mathé JF, et al. Acute spinal cord injury: Early care and treatment in a multicenter study with gacyclidine. *Soc Neurosci Abstr* 1999;25(Part 1):1090.
107. Tarlov I. Spinal cord compression studies. III. Time limits for recovery after gradual compression in dogs. *Arch Neurol* 1954;71:588–597.
108. Tator CH, Fehlings MG. Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. *J Neurosurg* 1991;75:15–26.
109. Thomas RJ. Excitatory amino acids in health and disease. *J Am Geriatr Soc* 1995;43:1279–1289.
110. Vignon J, Lazdunski M. Structure-function relationships in the inhibition of synaptosomal dopamine uptake by phencyclidine and analogues: Potential correlation with a binding site identified with [^{3}H]phencyclidine. *Biochem Pharmacol* 1984;33:700–702.
111. Vignon J, Privat A, Chaudieu I, Thierry A, Kamenka JM, Chicheportiche R. [^{3}H]Thienyl-phencyclidine ([^{3}H]TCP) binds to two different sites in rat brain. Localization by autoradiographic and biochemical techniques. *Brain Res* 1986;378:133–141.
112. Vincent JP, Cavey D, Kamenka JM, Geneste P, Lazdunski M. Interaction of phencyclidines with the muscarinic and opiate receptors in the central nervous system. *Brain Res* 1978;152:176–182.
113. Vincent JP, Kartalovski B, Geneste P, Kamenka JM, Lazdunski M. Interaction of phencyclidine ("angel dust") with a specific receptor in rat brain membranes. *Proc Natl Acad Sci USA* 1979;76:4678–4682.
114. Watanabe M, Inoue Y, Sakimura K, Mishina M. Distinct spatio-temporal distributions of the NMDA receptor channel subunit mRNAs in the brain. *Ann NY Acad Sci* 1993;707:463–466.
115. Watson BD, Dietrich WD, Busto R, Wachtel MS, Ginsberg MD. Induction of reproducible brain infarction by photochemically initiated thrombosis. *Ann Neurol* 1985;17:497–504.
116. Watson BD, Prado R, Dietrich WD, Ginsberg MD, Green BA. Photochemically induced spinal cord injury in the rat. *Brain Res* 1986;367:296–300.
117. Wrathall JR, Pettegrew RK, Harvey F. Spinal cord contusion in the rat: Production of graded, reproducible, injury groups. *Exp Neurol* 1985;88:108–122.