British Journal of Pharmacology (1996) 117, 1059–1064

Differential action of NMDA antagonists on cholinergic neurotoxicity produced by N-methyl-D-aspartate and quinolinic acid

A.C. Pawley, S. Flesher, R.J. Boegman, *R.J. Beninger & [1]K.H. Jhamandas

Department of Pharmacology and Toxicology and *Psychology, Queen's University, Kingston, Ontario, Canada K7L 3N6

1 Injections of N-methyl-D-aspartate (NMDA) and quinolinic acid (Quin), agonists that activate NMDA receptors, into the rat nucleus basalis magnocellularis (nbM) produced a dose-related decrease in cholineacetyltransferase (ChAT) activity in the cerebral cortex and amygdala 7 days after injection.

2 In order to examine the possibility that NMDA and Quin activate different sub-types of NMDA receptors to produce central cholinergic neurotoxicity, the sensitivity of these agonists to the action of three different NMDA receptor antagonists, 2-amino-7-phosphonoheptanoate (AP-7), 7-chlorokynurenate and dizolcipine (MK801) was examined by injecting a fixed dose of NMDA (60 nmol) or Quin (120 nmol) in combination with different doses of the antagonists into the nbM.

3 Both AP-7 (0.6–15 nmol) and 7-chlorokynurenate (3.75–200 nmol), which block the NMDA receptor recognition site and glycine modulatory site respectively, produced a dose-related attenuation of the NMDA or Quin-induced decrease in ChAT activity in both the cortex and amygdala. Both antagonists showed a greater potency against the action of NMDA than against Quin.

4 MK801 (2–200 nmol), an NMDA receptor-linked channel blocker, attenuated the Quin and NMDA response only at a high dose. Unlike AP-7 and 7-chlorokynurenate, MK801 did not exhibit a consistent difference in its potency as an antagonist against NMDA and Quin.

5 The differential antagonist actions of AP-7 or 7-chlorokynurenate against NMDA and Quin-induced cholinergic neurotoxicity suggest that the excitotoxic actions of these two agonists are mediated via distinct NMDA receptor sub-types. The NMDA- and Quin-sensitive receptors appear to differ with respect to properties of the receptor recognition and glycine modulatory sites that are associated with these receptors.

Keywords: Nucleus basalis; N-methyl-D-aspartate (NMDA); quinolinic acid (Quin); NMDA receptor antagonists; neurotoxicity

Introduction

Excitatory amino acids (EAA) such as glutamic acid act on specific receptors to produce synaptic excitation. Excessive neuronal activation by EAA, however, results in sustained depolarization and cell death, a phenomenon which has been designated as excitotoxicity. One of the major EAA receptor types implicated in excitotoxicity is the N-methyl-D-aspartate (NMDA) receptor (Rothman & Olney, 1987; Olney, 1990), a protein complex at which agonists such as NMDA or quinolinic acid (Quin) act to produce synaptic excitation and excitotoxicity. The NMDA receptor complex incorporates several distinct sites, a ligand recognition site, a strychnine-insensitive glycine site (Johnson & Ascher, 1987) and a cation channel site (Lodge *et al.*, 1988), at which specific ligands can act to influence the function of the receptor. Both pharmacological (Perkins & Stone, 1983a,b; French-Mullen *et al.*, 1986) and molecular biology studies (Monyer *et al.*, 1992; Meguro *et al.*, 1992; Nakanishi, 1992) have provided evidence for NMDA receptor heterogeneity. Recent studies have suggested that NMDA receptor subtypes may differ with respect to the pharmacological properties of distinct sites associated with the NMDA receptor complex (Monaghan *et al.*, 1988; Sekiguchi *et al.*, 1990).

Although the functional significance of NMDA receptor heterogeneity is not fully understood, neurotoxicity experiments involving the use of different NMDA receptor agonists such as ibotenic acid, NMDA and Quin have shown that different NMDA subtypes may be involved in the production of excitotoxic damage in the brain. EAA antagonists such as 2-amino-5-phosphonovalerate (AP-5), a blocker of the NMDA receptor recognition site, and kynurenic acid, a non-selective EAA receptor antagonist, have been found to exert differential action against Quin, NMDA or ibotenate-induced excitotoxicity (Foster *et al.*, 1984; Winn *et al.*, 1991).

If different NMDA receptor subtypes indeed mediate the excitotoxic response, such receptor subtypes may differ with respect to properties of some of the distinct sites associated with the NMDA receptor protein complex. The availability of site-selective NMDA receptor antagonists provides an opportunity to determine whether agonists such as NMDA and Quin activate different receptor subtypes to produce neurotoxicity. To examine this possibility the action of three distinct anti-NMDA agents, which either block the receptor recognition site 2-amino-7-phosphonoheptanoate (AP-7), the glycine modulatory site (7-chlorokynurenate) or the cation channel site (MK801), on the excitotoxic response produced by focal injections of NMDA and Quin has been investigated in a model of cholinergic neurotoxicity. The neurotoxic response measured in the present study involves reduction in the activity of cholineacetyltransferase (ChAT) in the cerebral cortex and amygdala, following injection of NMDA receptor agonists into the nucleus basalis magnocellularis (nbM). Previous studies, including those from this laboratory, have demonstrated that excitotoxic damage to large cholinergic neurones in the nbM, resulting in loss of cholinergic projections to cortex and amygdala, is reflected in depletion of regional ChAT activity (El-Defrawy *et al.*, 1985; Dunnett *et al.*, 1987; Boegman *et al.*, 1992). Thus, in this study the action of three distinct NMDA receptor antagonists on the NMDA and Quin-induced decrease in cortical and amygdala ChAT activity was examined

[1] Author for correspondence

Methods

Male Sprague Dawley rats weighing between 250–350 g were anaesthetized with 4% halothane in oxygen and maintained on 2% halothane during surgery for focal drug injections. The animal's head was placed in a stereotaxic frame and a single injection of agent under study was delivered into the nbM via a steel cannula (0.36 mm outer diameter). The stereotaxic coordinates for injection into the nbM were: 2.6 mm lateral to the midline, 0.8 mm posterior to bregma and 8.0 mm ventral to the surface of the skull (Paxinos & Watson, 1982). The incisor bar was set at −3.3 mm. The drug infusion, made into the right nbM, involved delivery of a 0.5 μl volume over a 70-s period. The injection cannula was left in place for an additional 2 min to allow for diffusion of the drug to the target site. Solutions of agents under study were made with 0.9% saline and adjusted to pH 7.4 with NaOH. In co-injection experiments, the excitotoxin and antagonist under study were dissolved in the same solution and delivered as a single infusion. After injection, the animal was allowed to recover from anaesthesia and subsequently returned to its home cage. Seven days following the focal injection, the animal was killed by guillotine and brain tissue from injected and uninjected sides was removed for biochemical determination of ChAT activity (see below). In some experiments the entire brain was removed for a histological verification of cannula placement in the nbM.

The frontoparietal cortex and amygdala were dissected and used for determination of ChAT activity as described in an earlier study (Boegman *et al.*, 1992). The cortical tissue was obtained by placing one razor blade at the optic chiasm and a second blade 4 mm caudal to this. A 4 mm thick coronal slice was removed bilaterally. The amygdala was obtained from a 2 mm wide coronal slice by freehand dissection. The tissues were rapidly homogenized for measurement of protein content by the method of Lowry *et al.* (1951), and for ChAT activity by the radiochemical method originally described by Fonnum (1975). The ChAT activity was expressed as nmol ACh formed per mg protein per hour. The cortical or amygdala ChAT activity measured in tissue from injected and uninjected hemispheres were compared and the change in enzyme activity produced by agents under study was expressed as percentage change relative to the uninjected side.

In selected experiments, slices of nbM were stained for specific acetylcholinesterase (AChE) activity to visualize damage to cholinergic neurones in the nbM. It has been shown that this damage can result in loss of AChE stain in the nbM (Boegman *et al.*, 1992). The animals were injected with diisopropylfluorophosphate (DFP) (1.8 mg kg^{-1}) 4–5 h before perfusion with paraformaldehyde. It has been shown previously that this pretreatment enhances the visualization of AChE positive cells (Parent & Butcher, 1976). The animals were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p.) and perfused transcardially with saline, followed by cold phosphate buffer containing 4% paraformaldehyde. The brains were then removed and post-fixed overnight in the same fixative. They were then immersed in 30% sucrose for at least 24 h. Transverse coronal sections (45 μm) were then cut on a freezing microtome and stored in individual wells containing 0.9% saline. Brain slices were stained for AChE containing neurones using the method described by Karnovsky & Roots (1964).

Drugs and chemicals

Quin and NMDA were obtained from Sigma Chemical Company, St. Louis, MI, U.S.A. 7-CKA and MK801 were obtained from RBI, Natick, MA, U.S.A. AP-7 was purchased from Tocris Cookson, Bristol, England. Halothane was supplied by Bensen, Markham, ON, Canada. All chemicals for histochemistry were supplied by Sigma Chemical Co. (St. Louis, Missouri, U.S.A.).

Statistical analysis

The dose-response relationship in the cortex and amygdala was assessed by a one way analysis of variance (ANOVA) ($P<0.05$). Differences in the effect of different agents on ChAT activity in the cortex or amygdala were assessed by Student's unpaired t test ($P<0.05$).

Results

Prior to study of antagonism of NMDA and Quin-induced neurotoxicity, the effect of saline injection and the dose-response effects of these agonists alone on nbM cholinergic neurones were investigated following single intra-nbM injections. Injection of saline into the nbM produced 3.9±1% and 12.8±5% ($n=9$) decrease in ChAT activity in the cortex and amygdala, respectively. Intra-nbM injections of NMDA or Quin produced dose-related decreases in cortical and amygdala ChAT activity measured seven days following a single injection (Figure 1). The dose-response curve for the action of NMDA on cortical ChAT activity was shifted to the left of that for Quin reflecting a greater potency of NMDA on the cortical cholinergic projection. However, the dose-response curves for the action of the two agonists on amygdala ChAT activity showed an overlap, indicating a similar potency of agonist action on the cholinergic projection to the amygdala. At doses of 60 nmol NMDA and 120 nmol Quin a comparable reduction in the cortical ChAT activity (45%) was observed. At these doses the two agonists also produced a comparable reduction in amygdala ChAT activity (60%). Thus these doses of NMDA and Quin were used in subsequent experiments to evaluate the comparative effects of different NMDA antagonists on the cholinergic neurotoxic response elicited by intra-nbM injections of NMDA or Quin.

Effect of AP-7

Figure 2 shows results of experiments in which different doses of AP-7 (0.6–15 nmol) were combined with a fixed dose of NMDA (60 nmol) or Quin (120 nmol) and injected into the nbM. Cortical and amygdala ChAT activity was measured seven days following injections. The antagonist AP-7 produced a dose-related inhibition of the NMDA and Quin effect in both

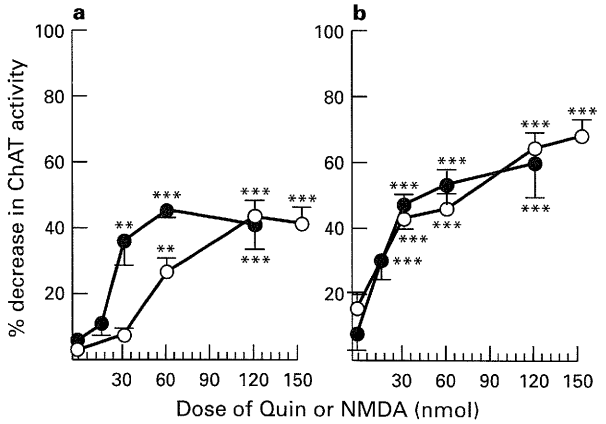

Figure 1 Dose-response curve for the decrease in cholineacetyltransferase activity in the cortex (a) and amygdala (b) observed 7 days following infusions of saline, N-methyl-D-aspartate (●, NMDA) or quinolinic acid (○, Quin) into the right nucleus basalis magnocellularis. Values shown are expressed as percentage decrease in enzyme activity in the cortex or amygdala relative to the corresponding region in the uninjected side in the same animal. Each point represents mean±s.e.mean; $n=5-6$ animals. $^{**}P<0.01$, $^{***}P<0.001$, values significantly different from saline-injected animals.

the cortex and amygdala. In both areas, the dose-response curve for the AP-7/NMDA combination was shifted to the left of that for the AP-7/Quin combination. Thus, AP-7 was more potent as an antagonist against NMDA than against Quin. The maximal inhibition of the NMDA and Quin effect by AP-7 in the cortex was apparent at the 2.5 and 7.5 nmol dose, respectively. In the amygdala a maximal inhibition of NMDA and Quin effects was seen at doses of 2.5 and 15 nmol, respectively. Thus, in both areas the dose of AP-7 that blocked the action of NMDA was lower than that producing inhibition of the Quin effect.

Effect of 7-chlorokynurenate

Figure 3 illustrates comparative effects of the glycine site antagonist, 7-chlorokynurenate (3.75–200 nmol), on reductions in cortical and amygdala ChAT produced by intra-nbM injections of NMDA or Quin. In both areas this agent produced a dose-related attenuation of NMDA and Quin effects. As was seen in the AP-7 experiments, the dose-response curve for the action of 7-chlorokynurenate action against NMDA was displaced to the left of that for its action against Quin. This leftward shift of the dose-response curve was especially prominent in the amygdala. However, high doses of 7-chlorokynurenate completely blocked the action of both agonists on ChAT activity. The maximal effect of 7-chlorokynurenate on the action of NMDA and Quin in the cortex was apparent at the 30 nmol dose. In the amygdala this effect against NMDA and Quin was seen at doses of 30 and 200 nmol, respectively. Thus, like AP-7, 7-chlorokynurenate showed a greater potency of action against NMDA than against Quin.

Effect of MK801

The effects of different doses of the channel blocker MK801 on the changes in ChAT activity produced by NMDA and Quin injections into the nbM are represented in Figure 4. In both areas MK801 (5–200 nmol) produced an apparent dose-related attenuation of NMDA and Quin effect on ChAT activity. However, a statistically significant action of MK801 was only observed after injection of a high dose. A significant inhibition of the action of NMDA and Quin action on ChAT activity in the cortex was seen at 60 and 25 nmol MK801, respectively. In the amygdala, the antagonism of the action of NMDA and Quin was seen at MK801 doses of 60 and 200 nmol, respectively. It is noteworthy that it was difficult to maintain high doses of MK801 in solution at pH 7.4, and the pH of the injected solution had to be adjusted to 5.5. The injection of the vehicle (0.5 μl, pH 5.5) alone produced no significant effect on ChAT activity (not shown).

Histological examination

The staining of AChE which occurs post DFP treatment has previously been shown to be associated with cholinergic neurones (Parent & Butcher, 1976). In this study, AChE staining was used to determine if there was a loss of neurones in the nbM following excitoxin injections in this region.

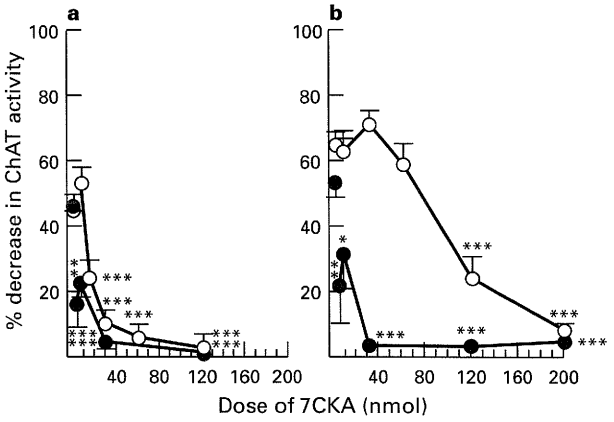

Figure 3 Dose-response curves for the effect of 7-chlorokynurenic acid (7-CKA) on the decrease in cholineacetyltransferase (ChAT) activity in the cortex (a) and amygdala (b) produced by unilateral injections of N-methyl-D-aspartate (●, 60 nmol) or quinolinic acid (○, 120 nmol). Values shown are expressed as percentage decrease in ChAT activity relative to the corresponding region in the uninjected side in the same animal. Each point represents mean ± s.e.mean; $n=4-5$ animals; $^{**}P<0.01$, $^{***}P<0.001$, indicates significantly different from value in absence of 7-CKA.

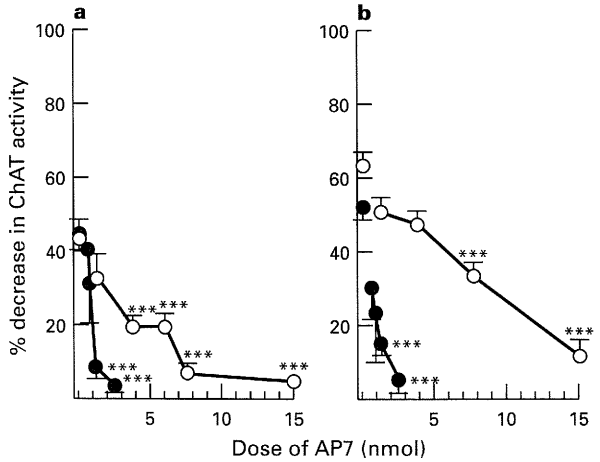

Figure 2 Dose-response curves for the effect of 2-amino-7-phosphonoheptanoate (AP-7) on the decrease in cholineacetyltransferase (ChAT) activity in the cortex (a) and amygdala (b) produced by unilateral injections of N-methyl-D-aspartate (●, 60 nmol) or quinolinic acid (○, 120 nmol). Values shown are expressed as percentage decrease in ChAT activity relative to the corresponding region in the uninjected side in the same animal. Each point represents mean ± s.e.mean; $n=4-10$ animals; $^{***}P<0.001$, indicates significantly different from value in the absence of AP-7.

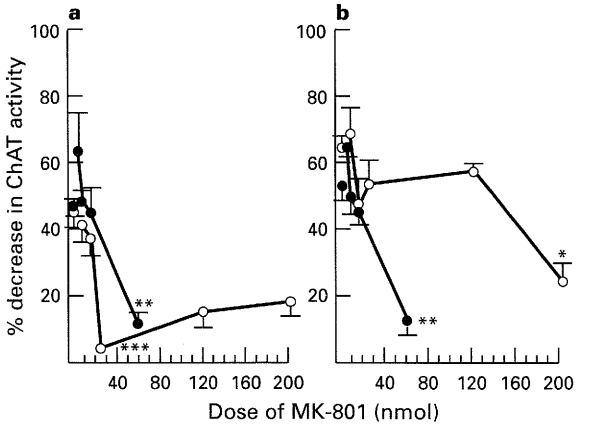

Figure 4 Dose-response curves for the effect of MK801 on the decrease in cholineacetyltransferase (ChAT) activity in the cortex (a) and amygdala (b) produced by unilateral injections of N-methyl-D-aspartate (●, 60 nmol) or quinolinic acid (○, 120 nmol). Values shown are expressed as percentage decrease in ChAT activity relative to the corresponding region in uninjected side in the same animal. Each point represents mean ± s.e.mean; $n=3-4$ animals; $^{**}P<0.01$, $^{***}P<0.001$ indicates significantly different from value in absence of MK801.

Examination of AChE stained sections thus allowed visualization of large cholinergic neurones in the nbM in animals which had been injected with saline (control group). In sections incorporating the nbM from animals given injection of 120 nmol Quin, there was almost complete loss of neurones staining for AChE. A similar loss of neurones was apparent in the nbM of animals receiving injections of 60 nmol NMDA but not saline. Injections of 30 nmol of 7-chlorokynurenate in combination with 120 nmol Quin resulted in partial sparing of AChE-positive neurones, while injection of this dose of antagonist with 60 nmol NMDA resulted in near complete sparing of neurones in the nbM. In all experiments involving unilateral Quin or NMDA injections, no damage to the contralateral side was observed (data not shown).

Discussion

The decrease in ChAT activity following injections of NMDA and Quin into the nbM serves as an index of the neurotoxic action of these agonists on cholinergic neurones projecting from nbM to the cerebral cortex or amygdala (Dunnett *et al.*, 1987; Boegman *et al.*, 1992). Previous studies have suggested that the cholinergic neurones projecting to the two regions are distinct (Boegman *et al.*, 1992; Heckers *et al.*, 1994). The results of NMDA antagonist experiments revealed a differential antagonism of NMDA and Quin-induced cholinergic neurotoxicity by the two distinct anti-NMDA agents, AP-7 and 7-chlorokynurenic acid. This finding suggests that the actions of NMDA and Quin resulting in cholinergic neurone damage, are mediated by subtypes of NMDA receptors. The difference in antagonist potency most likely reflects differences in the properties of the AP-7 and 7-chlorokynurenate-sensitive sites which are associated with the NMDA receptor subtypes activated by NMDA and Quin. As differential antagonism was seen both in the cortex and amygdala, these two NMDA receptor subtypes appear to be associated with both cholinergic projections originating from the nbM.

In a previous study of nbM involving antagonism of neurotoxicity produced by Quin, NMDA and ibotenic acid by AP-5 (which acts like AP-7), Winn *et al.* (1991) reported a differential antagonism of the action of Quin and ibotenate but not of Quin and NMDA by this agent. However, in the present study using AP-7, the action of Quin was distinguishable from that of NMDA on the basis of its lower sensitivity to this antagonist. The discrepancy between the results of the two studies may be related to different methods used to assess neurotoxicity. Winn *et al.* (1991) used the volume of the nbM lesion as a measure of neurotoxicity whereas the present study, which focused on damage to cholinergic neurones in the nbM, employed a decrease in cortical or amygdala ChAT activity as an index of toxicity. Previous studies have demonstrated that changes in this biochemical marker indeed result from damage to cholinergic neurones in the nbM (El-Defrawy *et al.*, 1985; Dunnett *et al.*, 1987; Boegman *et al.*, 1992). The greater antagonist potency of AP-7 action on the NMDA than on Quin-induced response suggests that the receptor recognition site to which NMDA binds has a higher affinity for AP-7 than that to which Quin binds to produce neurotoxicity.

The anti-NMDA agent, 7-chlorokynurenate, which blocks the strychnine-insensitive glycine site associated with the NMDA receptor complex, was also more potent in blocking NMDA than Quin-induced neurotoxicity. This potency difference was especially prominent for cholinergic projections to the amygdala. Previously, Winn *et al.* (1991) have reported that low doses of kynurenic acid, an agent which also blocks the glycine site but is less elective than 7-chlorokynurenate (Kemp *et al.*, 1988; Kessler *et al.*, 1989), were more effective in blocking NMDA- than Quin-induced neurotoxicity. However, higher doses of kynurenic acid were found by Winn *et al.* (1991) to be more effective against the action of Quin. In the present study, in which several doses of 7-chlorokynurenate were examined, the dose-dependent reversal of potency was not observed. Examination of the dose-response curves showed 7-chlorokynurenate to be a consistently more potent blocker of the action of NMDA than Quin. It would thus appear that the 7-chlorokynurenate-sensitive glycine site associated with the NMDA-sensitive receptor has a higher affinity for the antagonist than that associated with the Quin-sensitive NMDA receptor.

The channel antagonist MK801, unlike AP-7 and 7-chlorokynurenate, did not show a consistent difference in the potency of antagonist action against the NMDA and Quin-induced decrease in ChAT activity. A significant blockade of the two NMDA receptor agonists was only seen at a high dose of MK801 and the regional consistency of action seen in experiments with AP-7 and 7-chlorokynurenate was not seen in the MK801 experiments. As MK801 is an open channel blocker, its differential action against various NMDA receptor agonists may result more from differences in the functional state of the cation channel than from potential differences in properties of the MK801 binding sites. Interestingly, in experiments involving expression of cerebral and cerebellar NMDA receptor types in *Xenopus* oocytes, the channel blocker phencyclidine (PCP), which shares the action of MK801, has not been as useful as 7-chlorokynurenate in distinguishing between these receptors (Sekiguchi *et al.*, 1990). Thus, in this study MK801 was not as useful as the other two antagonists in distinguishing between the actions of NMDA and Quin. It should be noted that MK801 was relatively less effective in blocking Quin-induced neurotoxicity, especially in the amygdala. In a previous study MK801 was reported to block completely the action of Quin on striatal cholinergic neurones (Foster *et al.*, 1988). However, in that study MK801 was given systemically as pretreatment and the neuronal population (cholinergic interneurones) targeted by MK801 was different from that in the present study. These factors may partly explain the differences in the effectiveness of MK801 in various brain regions.

The differential effects of the two NMDA antagonists appear not to result from variable levels of receptor activation by NMDA and Quin since doses of the two agonists used here produced similar responses (loss of ChAT activity) in the cerebral cortex and in the amygdala. In the cortex, 120 nmol Quin and 60 nmol NMDA were equieffective, producing maximal depletion of ChAT activity. In the amygdala these doses also produced a maximal depletion of ChAT activity. The magnitude of effect produced by 60 and 120 nmol Quin in the amygdala was not statistically significant. However, the higher dose was used since it allowed investigation of antagonist action against equieffective doses of Quin and NMDA on the two cholinergic pathways in the same animal. This experimental approach minimized the influence of extrinsic factors, such as anaesthesia, stereotaxic injections and tissue dissection, on the neurotoxic response in two pathways which originate from the same brain region. The relative resistance of amygdala to antagonist action may depend on certain distinct neurochemical characteristics of cholinergic neurones projecting to this area (Heckers *et al.*, 1994).

Previous studies involving [^{3}H]-glutamate binding to different brain regions or expression of NMDA receptors in *Xenopus* oocytes have identified cerebellar and cerebral NMDA receptor subtypes distinguishable on the basis of their sensitivity to glycine. In [^{3}H]-glutamate binding studies, Monaghan *et al.* (1988) found that the cerebellar type of NMDA receptor was resistant to glycine. Sekiguchi *et al.* (1990) reported that in electrophysiological experiments on *Xenopus* oocytes expressing cerebellar and cerebral NMDA receptors, the former were resistant to blockade by 7-chlorokynurenate. Thus, in the present study a relative resistance to 7-chlorokynurenate of Quin-sensitive receptors might suggest that these receptors correspond to the cerebellar subtype of NMDA receptor originally described by Monaghan *et al.* (1988). However, other observations tend to argue against this notion. Monaghan & Beaton (1991) showed that the cerebellar NMDA receptor is Quin-insensitive. Additionally, Sekiguchi

et al. (1990) could not readily differentiate between the cerebellar and cerebral type on the basis of sensitivity to D-APV. In the present study the Quin-sensitive receptor was considerably less sensitive to AP-7, an agent which shares the action of D-APV. Thus, the Quin-sensitive receptor mediating cholinergic neurotoxicity, while being relatively resistant to 7-chlorokynurenate blockade, does not appear to correspond to the cerebellar NMDA receptor described in other studies. Recently, molecular biology studies have identified a number of NMDA receptor types. However, the relationship of the NMDA and Quin-sensitive receptors mediating neurotoxicity to these receptor types is not known.

The apparent potencies of Quin and NMDA with regard to the neurotoxic response were similar while Quin has been reported to be much weaker than NMDA in receptor binding experiments (Patneau & Mayer, 1990). This discrepancy may be due to other factors which influence Quin-induced neurotoxicity. The neurotoxic action of Quin, but not NMDA, requires the presence of presynaptic glutamatergic afferents to target neurones (Schwarcz *et al.*, 1984). Thus, the level of glutamatergic input to cholinergic neurones may be a factor in the discrepancy between potencies of Quin and NMDA *in vitro* and *in vivo*.

The difference between the actions of NMDA antagonists seen in previous studies and also observed in this investigation has implications for the use of these agents as neuroprotectants. Excitotoxicity, involving excessive NMDA receptor activation is thought to be a contributory mechanism in the neurone loss apparent in acute hypoxic-ischaemic brain damage (Rothman & Olney, 1986) or in certain chronic neurodegenerative diseases (Schwarcz *et al.*, 1984; Choi, 1988), and NMDA receptor antagonists are considered to be potentially useful in limiting or preventing this neurone loss. The results of the present study show that the success of NMDA receptor type antagonism may depend on the NMDA receptor sub-type activated during the pathophysiological event involving excitotoxic damage. Excitotoxicity involving activation of Quin-sensitive receptors may respond less readily to agents such as AP-7 and 7-chlorokynurenate than excitotoxicity involving NMDA-sensitive receptors. Alternatively, large doses of such antagonists may have to be administered in order to block effectively different sub-types of NMDA receptors.

The authors acknowledge financial support of the Medical Research Council of Canada, and thank Ms B. Ison for assistance in preparing the manuscript.

References

BOEGMAN, R.J., COCKHILL, J., JHAMANDAS, K. & BENINGER, R.J. (1992). Excitotoxic lesions of rat basal forebrain: differential effects on choline acetyl transferase in the cortex and amygdala. *Neurosci.*, (Oxford), **51,** 129–135.

CHOI, D.W. (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron*, **1,** 623–634.

DUNNETT, S.B., WHISHAW, I.Q., JONES, G.H. & BUNCH, S.T. (1987). Behavioural, biochemical and histochemical effects of different neurotoxic amino acids injected into nucleus basalis magnocellularis of rats. *Neurosci.*, (Oxford), **20,** 653–669.

EL-DEFRAWY, S.R., COLOMA, F., JHAMANDAS, K., BOEGMAN, R.J., BENINGER, R.J. & WIRSCHING, B.A. (1985). Functional and neurochemical cortical cholinergic lesions of the nucleus basalis magnocellularis in the rat. *Neurobiol. Aging*, **6,** 325–330.

FFRENCH-MULLEN, J.M.H., HORI, N. & CARPENTER, D.O. (1986). A comparison of the effects of quinolinate and NMDA on neurons in rat piriform cortex. *Neurosci. Lett.*, **63,** 66–70.

FONNUM, F. (1975). A rapid radiochemical method for the determination of choline acetyltransferase. *J. Neurochem.*, **24,** 407–409.

FOSTER, A.C., GILL, R. & WOODRUFF, G.N. (1988). Neuroprotective effects of MK801 *in vivo*: selectivity and evidence for delayed degeneration mediated by NMDA receptor activation. *J. Neurosci.*, **8,** 4745–4754.

FOSTER, A.C., VEZZANI, A., FRENCH, E.D. & SCHWARCZ, R. (1984). Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. *Neurosci. Lett.*, **48,** 273–278.

HECKERS, S., OHTAKE, T., WILEY, R.G., LAPPI, D.A., GUELLA, C. & MESULAM, M.M. (1994). Complete selective cholinergic denervation of the rat neocortex and hippocampus but not amygdala by an immunotoxin against p75NGF receptor. *J. Neurosci.*, **14,** 1271–1289.

JOHNSON, J.W. & ASCHER, P. (1987). Glycine potentiates the N-methyl-D-aspartate response in cultured mouse brain neurons. *Nature*, **325,** 529–531.

KARNOVSKY, M.J. & ROOTS, L. (1964). A 'direct colouring' thiocholine method for cholinesterases. *J. Histochem. Cytochem.*, **12,** 219–221.

KEMP, J.A., FOSTER, A.C., LEESON, P.D., PRIESTLY, T., TRIDGETT, R., IVERSEN, L.L. & WOODRUFF, G.S. (1988). 7-Chlorokynurenic acid is a selective antagonist at glycine modulatory site of the N-methyl-D-aspartate receptor complex. *Proc. Natl. Acad. Sci. U.S.A.*, **85,** 6547–6550.

KESSLER, M., TERRAMANI, T., LYNCH, G. & BAUDRY, M. (1989). A glycine site associated with *N*-methyl-D-aspartic acid receptors: Characterization and identification of a new class of antagonists. *J. Neurochem.*, **52,** 1319–1328.

LODGE, D., ARAM, J.A., CHURCH, J., DAVIES, S.N., MARTIN, D., MILLAR, J. & ZEMAN, S. (1988). Sigma opiates and excitatory amino acids. In *Excitatory Amino Acids in Health Disease* ed. Lodge, D. pp. 237–259. Chichester: Wiley & Sons Ltd.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.S. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193,** 265–275.

MEGURO, H., MORI, H., ARAKI, L., KUSHIYA, E., KUTSUWADA, T., YAMAZAKI, M., KUMANISHI, T., ARAKAWA, M., SAKIMURA, K. & MISHINA, M. (1992). Functional characterization of a heterometric NMDA receptor channel expressed from cloned cDNAs. *Nature*, **357,** 70–74.

MONAGHAN, D.T. & BEATON, J.A. (1991). Quinolinate differentiates between forebrain and cerebellar NMDA receptors. *Eur. J. Pharmacol.*, **194,** 123–125.

MONAGHAN, D.T., OLIVERMAN, H.J., NGUYEN, L., WATKINS, J.C. & COTMAN, C.W. (1988). Two classes of N-methyl-D-aspartate recognition sites: Differential distribution and differential regulation by glycine. *Proc. Natl.Acad. Sci. U.S.A.*, **85,** 9836–9840.

MONYER, H., SPRENGEL, R., SCHOEPFER, R., HERB, A., HIGUCHI, M., LOMELI, H., BURNASHEV, N., SAKMANN, B. & SEEBURG, P.H. (1992). Heterometric NMDA receptors–molecular and functional distinction of subtypes. *Science*, **256,** 1217–1221.

NAKANISHI, S. (1992). Molecular diversity of glutamate receptors and implications for brain function. *Science* (Washington, D.C.), **258,** 597–603.

OLNEY, J.W. (1990). Excitotoxic amino acids and neuropsychiatric disorders. *Annu. Rev. Pharmacol. Toxicol.*, **30,** 47–71.

PARENT, A. & BUTCHER, L.L. (1976). Organization and morphologies of acetylcholinesterase containing neurons in the thalamus and hypothalamus of the rat. *J. Comp. Neurol.*, **170,** 205–226.

PATNEAU, D.K. & MAYER, M.L. (1990). Structure-activity relationships for amino acid transmitter candidates acting at N-Methyl-D-aspartate and Quisqualate receptors. *J. Neurosci.*, **10,** 2385–2399.

PAXINOS, G. & WATSON, C. (1982). *The Rat Brain in Stereotaxic Coordinates.* 1st Edition. Sydney, Australia: Academic Press.

PERKINS, M.N. & STONE, T.W. (1983a). Quinolinic acid: regional variations in neuronal sensitivity. *Brain Res.*, **259,** 172–176.

PERKINS, M.N. & STONE, T.W. (1983b). Pharmacology and regional variations of quinolinic acid-induced excitations in the rat central nervous system. *J. Pharmacol. Exp. Ther.*, **226,** 551–557.

ROTHMAN, S.M. & OLNEY, J.W. (1987). Excitotoxicity and the N-methyl-D-aspartate receptor. *Trends Neurosci.*, **20,** 299–302.

ROTHMAN, S.M. & OLNEY, J.W. (1986). Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Ann. Neurol.*, **19,** 105–111.

SCHWARCZ, R., FOSTER, A.C., FRENCH, E.D., WHETSELL, JR. W.O. & KÖHLER, C. (1984). Excitotoxic models of neurodegenerative disorders. *Life Sci.*, **35,** 19–32.

SEKIGUCHI, M., OKAMOTO, K. & SAKAI, Y. (1990). Glycine-insensitive NMDA-sensitive receptor expressed in Xenopus oocytes by guinea pig cerebellar mRNA. *J. Neurosci.*, **10,** 2148–2155.

WINN, P., STONE, T.W., LATIMER, M., HASTINGS, M.H. & CLARK, A.J.M. (1991). A comparison of excitotoxic lesions of the basal forebrain by kainate, quinolinate, ibotenate, *N*-methyl-D-aspartate or quisqualate, and the effects on toxicity of 2-amino-5-phosphonovaleric acid and kynurenic acid in the rat. *Br. J. Pharmacol.*, **102,** 904–908.

(Received June 22, 1995
Revised November 12, 1995
Accepted November 14, 1995)