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Anticonvulsant activity of AMPA/kainate antagonists: comparison of GYKI 52466 and NBQX in maximal electroshock and chemoconvulsant seizure models

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The anticonvulsant activities of a noncompetitive (GYKI 52466) and a competitive (NBQX) AMPA/kainate antagonist were compared in the maximal electroshock (MES) seizure test and various chemoconvulsant models. Both antagonists were protective in the MES and pentylenetetrazol tests. GYKI 52466 was also protective against seizures and lethality induced by 4-aminopyridine, kainate and AMPA, but not by NMDA, whereas NBQX was ineffective in these chemoconvulsant tests. Both GYKI 52466 and NBQX produced motor impairment at doses similar to those that were protective in the MES test. Under some circumstances, noncompetitive AMPA/kainate antagonists could offer advantages over competitive antagonists in seizure therapy. However, neurological toxicity is an obstacle to the potential clinical use of both classes of agents.

Introduction

The recent availability of centrally active antagonists of non-NMDA (AMPA/kainate) excitatory amino acid receptors has made it possible to evaluate the potential of such compounds as antiepileptic agents in animal seizure models. The first selective AMPA/kainate antagonists to be described were quinoxalinediones[5], such as NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline) which is active centrally and does not block NMDA receptors[13]. More recently, the 2,3-benzodiazepine GYKI 52466 [1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine HCl][17] has been identified as a potent and highly selective antagonist of AMPA/kainate responses[6,11,18]. In contrast to the quinoxalinediones that block non-NMDA receptor responses by competing at the excitatory amino acid recognition site, GYKI 52466 is a noncompetitive antagonist that appears to act by a novel allosteric mechanism[6,22]. A key difference between competitive and noncompetitive non-NMDA antagonists is that the blocking action of competitive antagonists can be overcome by high levels of glutamate whereas the degree of block produced by noncompetitive antagonists is largely independent of agonist concentration.

Both NBQX and GYKI 52466 have been demonstrated to have anticonvulsant activity in reflex sei-

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zure models[1,2,14], and NBQX has been reported to have anticonvulsant in the maximal electroshock (MES) test[19] and various chemoconvulsant models[20]. In the present study, we compared the ability of GYKI 52466 and NBQX to protect against tonic hindlimb extension in the mouse MES test, against lethality induced by 4-aminopyridine (4-AP), and against seizures and lethality induced by selective excitatory amino acid receptor agonists. Our results confirm that both non-NMDA antagonists are effective broad spectrum anticonvulsants, but suggest that, in certain situations, noncompetitive antagonists such as GYKI 52466 could offer advantages over competitive antagonists in seizure therapy.

Methods

Animals

Male NIH Swiss mice (25–30 g) were obtained from the National Institutes of Health (NIH) Animal Program. Animals were allowed to acclimatize with free access to food and water for a 24-h period before testing. All procedures were carried out in strict compliance with the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the NIH Animal Use Committee.

MES seizure test

Fifteen minutes after i.p. injection of GYKI 52466 or 30 min after i.p. injection of NBQX (or at the indicated intervals in the time course experiment) the animals were subjected to a 0.2-s, 60-Hz, 50-mA electrical stimulus delivered with corneal electrodes (5 mm diameter stainless steel balls) wetted with 0.9% saline. Animals failing to show tonic hindlimb extension were scored as protected. The latencies between i.p. drug injection and testing were chosen based upon the times of peak effect in mice as reported by Chapman et al.[2].

PTZ seizure test

Compounds were evaluated for their ability to protect against s.c. PTZ (85 mg/kg)-induced clonic seizures according to the procedure described by Swinyard et al.[15]. PTZ was administered 15 min or 30 min after i.p. injection of GYKI 52466 or NBQX, respectively.

4-AP seizure test

The 4-AP seizure test was carried out as described by Yamaguchi and Rogawski[21]. In brief, a 13.3 mg/kg dose of 4-AP was injected s.c. 15 or 30 min after i.p. injection of GYKI 52466 or NBQX, respectively. The endpoint in this test was lethality.

AMPA, kainate and NMDA seizure tests

AMPA, kainate and NMDA were administered in doses of 200, 32 and 257 mg/kg, s.c. (previously determined ED_{97} values) 15 or 30 min after GYKI 52466 or NBQX, respectively. For AMPA, animals failing to show explosive running, jumping or barreling followed by continuous clonic limb movements were scored as protected. In the case of kainate, the endpoint was 5 s or more of clonic seizure activity, whereas for NMDA the endpoint was lethality. The observation period was 60 min.

Motor toxicity test

Evaluation for motor toxicity was carried out using a modification of the horizontal screen test described by Coughenour et al.[4] which determines an animal's ability to support its own body weight by grasping a grid. Untrained mice were placed on a horizontally oriented grid (consisting of parallel 1.5 mm diameter rods situated 1 cm apart) and the grid was inverted. Animals that fell from the grid within 5 s were scored as positive.

Drugs and solutions

Convulsant drugs were administered in a volume of 0.9% saline equalling 1% of the animal's body weight. GYKI 52466 and NBQX were administered in deionized water and were generous gifts, respectively, of Dr. I. Tarnawa (Institute for Drug Research, Hungary) and Drs. T. Honoré and L. Nordholm (Novo Nordisk A/S, Måløv, Denmark). AMPA was from Tocris Neuramin (Essex, UK). All other drugs were from Sigma Chemical Co. (St. Louis, MO, USA).

Data analysis

ED_{50} values (dose protective in 50% of animals) and their 95% confidence limits were determined by log-probit analysis using the Litchfield-Wilcoxon method[16]. Drugs failing to protect >50% of animals at the maximum dose tested were consid-

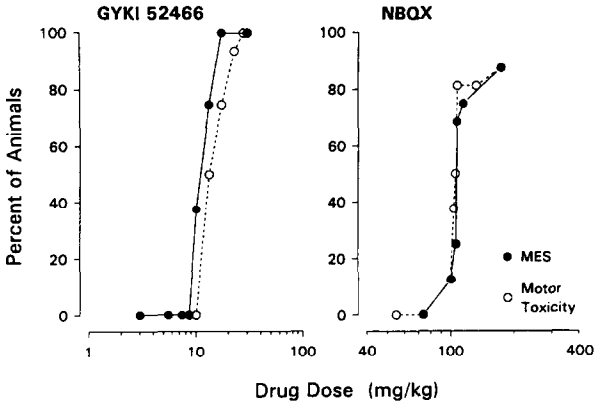

Fig. 1. Comparison of the dose-effect relationships for protection against MES seizures and induction of motor toxicity. Motor toxicity determinations were carried out immediately prior to the presentation of the MES stimulus. In this and subsequent figures, each data point represents eight mice.

ered ineffective. The dose causing motor impairment in 50% of animals is referred to as the TD_{50}. Using the data obtained in the MES seizure test, we calculated the therapeutic index (TI), a measure of relative toxicity, as the ratio TD_{50}/ED_{50}.

TABLE I

Anticonvulsant activity of GYKI 52466 and NBQX in anticonvulsant screening tests and excitatory amino acid seizures

Seizure model	ED_{50} (mg/kg, i.p.)	
	GYKI 52466	NBQX
Anticonvulsant screening tests		
MES[a]	11.8 (9.7–14.3)	125.6 (94.9–166.2)
PTZ[b]	22.5 (18.5–27.4)	85.9 (71.5–103.3)
4-AP[c]	96.5 (85.6–108.9)	> 173
Excitatory amino acid seizures		
NMDA[c]	> 30	> 173
AMPA[d]	23.0 (18.9–28.0)	> 173
Kainate[b]	8.4 (5.7–12.4)	> 173

[a]Tonic seizures. [b]Clonic seizures. [c]Lethality. [d]Wild running seizures.

Results

MES seizure test and toxicity test

As illustrated in Fig. 1, both GYKI 52466 and NBQX protected mice in a dose-dependent fashion against tonic hindlimb extension in the MES test. The ED_{50} values are summarized in Table I. The AMPA/kainate antagonists also caused motor impairment at doses similar to those that were protective in the MES test. The TD_{50} values for GYKI 52466 and NBQX were 13.2 (95% confidence limits: 11.2–15.6) and 113 (105–122) mg/kg, respectively, resulting in TI values of 1.1 and 0.9.

Time course for protection in MES test

The time course for protection in the MES test was determined following administration of doses of GYKI 52466 and NBQX equivalent to twice their ED_{50} values as determined in the dose-response experiments described above. At these doses, the protective effect of GYKI 52466 had worn off by 90 min following injection whereas the protective activity of NBQX persisted for > 120–240 min (Fig. 2).

Chemically induced seizures

Fig. 3 illustrates the dose-response curves for protection in the chemoconvulsant tests (for comparison, MES data from Fig. 1 are also shown). GYKI 52466 was protective against clonic seizures and lethality induced by PTZ and 4-AP as well as seizures induced by the excitatory amino acid agonists kainate and AMPA. In contrast,

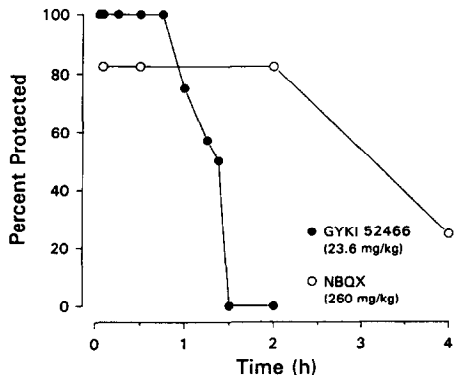

Fig. 2. Time courses for protection against MES seizures by GYKI 52466 and NBQX.

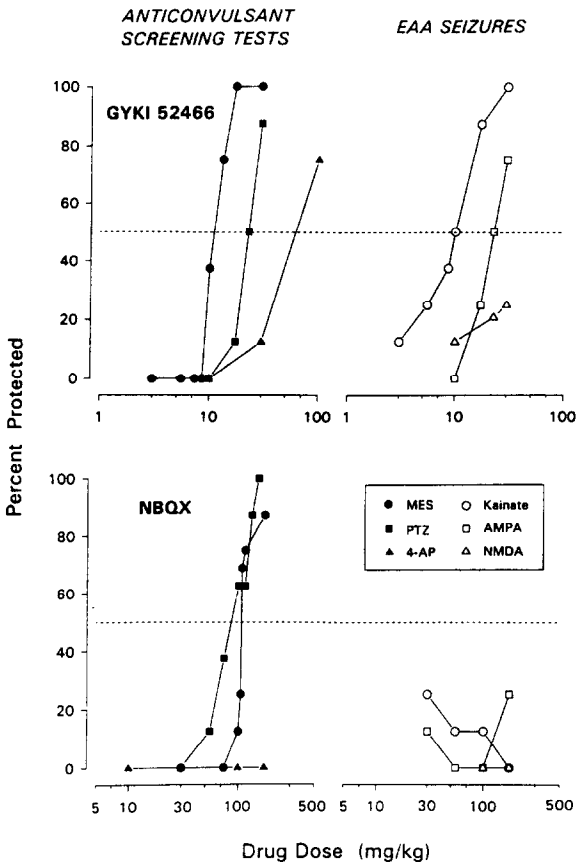

Fig. 3. Dose-effect relationships for protection in various seizure models by GYKI 52466 and NBQX. Test drugs were administered i.p. prior to the electroshock stimulus or s.c. injection of the chemoconvulsant. The 50% points are marked with a dotted line, but the ED_{50} values given in Table I were determined by log-probit analysis. MES data are taken from Fig. 1.

NBQX was protective in the PTZ test but not in the 4-AP test, and, under the conditions of these experiments, failed to protect against seizures induced by AMPA and kainate at doses up to 173 mg/kg. GYKI 52466 and NBQX failed to protect >50% of animals against NMDA-induced lethality at doses of 30 and 173 mg/kg, respectively.

Discussion

This study confirms the effectiveness of AMPA/kainate antagonists in the MES test, a widely used model for anticonvulsant drug screening[12]. The competitive AMPA/kainate antagonist NBQX has previously been demonstrated to be protective in this model[20], and, in the present study, we observed that the novel noncompetitive AMPA/kainate antagonist GYKI 52466 was similarly effective. However, for both drugs, the dose-response curves for seizure protection nearly overlapped those obtained in the motor toxicity test (Fig. 1), suggesting that neurological side effects may be an impediment to the clinical use of AMPA/kainate antagonists in seizure therapy. Nevertheless, in certain seizure types, most notably reflex (audiogenic) seizures in genetically epilepsy-prone animals[2,14,20] but also seizures induced by certain GABA antagonists or GABA-depleting drugs[20], AMPA/kainate antagonists are more potent so that there is greater separation between the anticonvulsant and toxic doses. However, the relevance of anticonvulsant activity in these models to clinical effectiveness in epilepsy therapy is not well established.

In contrast to its protective activity in the MES test, under the conditions used in these experiments, NBQX was ineffective against seizures and lethality induced by 4-AP (confirming our previous results[21]), whereas GYKI 52466 was active in this model. 4-AP causes a profound enhancement of neurotransmitter release at central and peripheral synapses, and in vitro studies[7,9] have suggested that excessive activation of central AMPA/kainate receptors is primarily responsible for the seizures and lethality produced by the drug in animals. Therefore, the ineffectiveness of NBQX in this model was unexpected[21]. A possible explanation for the failure of NBQX is that its blocking action is competitively overcome by high synaptic levels of glutamate that are presumably present during seizures in 4-AP treated animals. Doses of NBQX that are sufficient to adequately block AMPA/kainate receptors in 4-AP treated animals would excessively block AMPA/kainate receptors under normal conditions. Because of the critical role of AMPA/kainate receptors in brain function, excessive blockade of these receptors would presumably be highly toxic or lethal. In contrast, the AMPA/kainate receptor blocking action of the noncompetitive antagonist GYKI 52466 would not be overcome by high synaptic levels of glutamate and it would therefore exhibit anticonvulsant activity at relatively lower doses (with respect to the toxic dose).

In the kainate and AMPA models as in the 4-AP

model, NBQX lacked the protective activity of GYKI 52466. As in the 4-AP model, we propose that excessive receptor levels of kainate and AMPA consequent to their high dose systemic administration may overcome the block produced by NBQX. However, the failure of NBQX in the kainate and AMPA models is dependent upon the specific experimental conditions we used. In fact, Chapman et al.[2] have observed NBQX to be protective against seizures induced by intracerebroventricular AMPA whereas other workers, using different experimental paradigms, found, as we did, that NBQX is not effective (W. Danysz, personal communication). Thus, our results should not be interpreted as indicating that NBQX lacks AMPA/kainate receptor blocking activity in vivo, but rather that under certain experimental conditions the drug is relatively less effective than the noncompetitive antagonist GYKI 52466. Finally, both NBQX and GYKI 52466 were ineffective in protecting against NMDA-induced lethality as is consistent with their lack of NMDA receptor blocking activity[10].

In conclusion, the effectiveness of AMPA/kainate antagonists in standard anticonvulsant screening models provides further support for the potential utility of this class of excitatory amino acid receptor antagonist in epilepsy therapy. AMPA/kainate receptors play a critical role as mediators of excitatory neurotransmission in virtually all brain systems[3], and it is therefore not surprising that AMPA/kainate antagonists produce toxicity at anticonvulsant doses; whether this toxicity will be an impediment to the development of such compounds for clinical use remains to be determined. In contrast to the seizure models utilized in the present study, AMPA/kainate antagonists protect against audiogenic seizures in genetically epilepsy prone animals at substantially lower doses than those that cause neurological toxicity[2]. Moreover, in reflex seizure models, NBQX may be relatively more effective than GYKI 52466[2]. However, in the 4-AP, kainate and AMPA models, where seizures may be associated with excessive agonist activation of AMPA/kainate receptors, only the noncompetitive antagonist GYKI 52466 had protective activity (under the experimental conditions we used). Therefore, noncompetitive AMPA/kainate antagonists such as GYKI 52466 could be advantageous in the treatment of certain seizure types, particularly those associated with excessive glutamate release.

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