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In vivo pharmacology of BIIR 561 CL, a novel combined antagonist of AMPA receptors and voltage-dependent Na^+ channels

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1 Glutamate receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype and voltage-gated Na^+ channels are associated with diseases of the central nervous system characterized by neuronal over-excitation as in epilepsy or cerebral ischaemia. In animal models, AMPA receptor antagonists and Na^+ channel blockers provide protection in these conditions.

2 Dimethyl-{2-[2-(3-phenyl-[1,2,4]oxadiazol-5-yl)-phenoxyl]-ethyl}-amine hydrochloride (BIIR 561 CL) combines both, AMPA receptor–and Na^+ channel blocking properties in one molecule. Here, BIIR 561 CL was investigated *in vivo*.

3 BIIR 561 CL protected mice against AMPA-induced toxicity with an ED_{50} value of 4.5 mg kg^{-1} following subcutaneous (s.c.) administration. A 0.1% solution of BIIR 561 CL provided local anaesthesia in the corneal reflex test in rabbits. In mice, the compound prevented tonic seizures in the maximal electroshock (MES) model with an ED_{50} value of 3.0 mg kg^{-1} s.c. In amygdala-kindled rats, BIIR 561 CL inhibited seizures at doses of 3 and 11 mg kg^{-1} following intraperitoneal (i.p.) injection.

4 The data show that the combination of blocking AMPA receptor- and voltage-gated Na^+ channels in one molecule induces effective protection in animal models of neuronal over-excitation. *British Journal of Pharmacology* (2001) **133,** 789–796

Keywords: AMPA antagonist; Na^+ channel blocker; neuroprotection; local anaesthesia; anticonvulsion; kindling; *in vivo* pharmacology

Abbreviations: ADD, afterdischarge duration; ADT, afterdischarge threshold; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; aptiganel, N-(3-ethylphenyl)-N-methyl-N′-(1-naphthalenyl)-guanidine monohydrochloride; BIIR 561 CL, dimethyl-{2-[2-(3-phenyl-[1,2,4] oxadiazol-5-yl)-phenoxyl]-ethyl}-amine hydrochloride; CNS, central nervous system; EEG, electroencephalogram; GYKI 52466, 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine; i.p., intraperitoneal; i.v., intravenous; MES, maximal electroshock; NBQX, 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione; NMDA, N-methyl-D-aspartate; p.o., peroral; s.c., subcutaneous; SD, seizure duration; SS, seizure severity; TI, therapeutic index

Introduction

Neurodegeneration is thought to emerge as a consequence of the initiation of the excitotoxic cascade where excessive release of glutamate results in depolarization through activation of neuronal ionotropic glutamate receptors of the N-methyl-Da-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) types followed by activation of voltage-gated Na^+-, and Ca^{2+} channels (Olney, 1978). This process is supposed to play a major role in acute CNS diseases such as focal or global cerebral ischaemia, head trauma or in chronic diseases such as epilepsy and maybe even Alzheimer's disease and other chronic progredient degenerative diseases of the brain (Doble, 1995; Lees, 1996). Hence, drugs that interrupt the excitotoxic cascade by blocking glutamate-gated or voltage-gated channels are potentially neuroprotective in these conditions.

Since 1990, AMPA receptor antagonists that cross the blood–brain barrier have become available, and a number of studies have indicated that AMPA antagonists are more effective than NMDA receptor antagonists in preventing neuronal loss. While the competitive AMPA receptor antagonist 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX, Sheardown *et al*., 1990) suffered from low solubility causing nephrotoxicity (Gill, 1994; Xue, 1994), newer derivatives with better water-solubility have been developed and currently undergo clinical testing (Turski *et al*., 1998). 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzo-diazepine (GYKI 52466) is a non-competitive AMPA receptor antagonist and was first described as a muscle relaxant with anticonvulsive properties (Berzsenyi *et al*., 1988). However, low water-solubility and a small therapeutic window have limited its therapeutic potential. Adverse events, which may be expected from blocking AMPA receptors, are motor impairment, sedation and respiratory depression (Gill, 1994; Lees, 1996).

Voltage-dependent Na^+ channels have long been regarded as a target for anti-arrhythmic and local anaesthetic drugs, and have attracted additional attention in the mid-1980s as an important site of action of anticonvulsant drugs (for

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review see Taylor & Narasimhan, 1997). As these channels became also recognized as an essential element of the excitotoxic cascade, blockers of voltage-gated Na^+ channels became interesting drug candidates for neuroprotective treatment (Taylor & Meldrum, 1995). Adverse effects expected from Na^+ channel blockers are mainly cardiovascular in nature such as changes in heart rate and reduction of blood pressure (Taylor & Narasimhan, 1997). Since it was shown that blockers of Na^+ channels improve the efficacy of glutamate receptor antagonists in cell culture models of oxygen/glucose deprivation (Lynch *et al*., 1995), the combination of both mechanisms of action to improve efficacy appears as a logical conclusion.

BIIR 561 CL is a novel compound which combines a non-competitive block of AMPA receptors with an inhibition of currents flowing through voltage-gated Na^+ channels with comparable potency (Weiser *et al*., 1999). We describe the effects of BIIR 561 CL in models indicative of AMPA antagonistic-, local anaesthetic-, and anticonvulsant activity. The drug is as a promising candidate for anticonvulsant or even neuroprotective therapy.

Methods

AMPA-induced lethality

The method was adapted from Leander *et al*. (1988). Male albino mice (OF1, IFFA Credo, France) weighing 21–32 g were used. The animals had free access to a standard pellet diet and tap water in an air-conditioned animal room (23°C).

The test compound BIIR 561 CL, AMPA and the reference compounds N-(3-ethylphenyl)-N-methyl-N′-(1-naphthalenyl)-guanidine monohydrochloride (aptiganel), GYKI 52466 and mexiletine were dissolved in de-mineralized water. NBQX was dissolved in de-mineralized water with addition of one equivalent lithium hydroxide. Compounds were injected s.c. in an administration volume of 0.1 ml/10 g body weight 15 min before the administration of AMPA ($n=5-10$ per group). The mice were transferred to a restraining cage before AMPA was administered intravenously (i.v.) in a tail vein at a dose of 60 mg kg^{-1} in a volume of 0.1 ml/10 g body weight over 20 s. This dose was lethal within 1 min in 90–100% of control animals.

The proportion of animals surviving an observation period of 20 min following the AMPA infusion was taken as a measure of the compound's ability to protect against AMPA-induced lethality. Efficacy was expressed as ED_{50} value, and confidence limits were calculated by a probit analysis using the SAS software program system versions 6.08 and 6.11 (SAS Institute Inc., Cary, NC, U.S.A.).

Corneal reflex test

The experiments were performed in Chinchilla rabbits of either sex (Chbb:CH) weighing 3.2–4.1 kg. The animals were kept in individual cages in an air-conditioned room (23°C) with free access to tap water and a standard pellet diet. The light/dark cycle was 12 h. Animals had not been used for a corneal reflex test for at least 4 days. BIIR 561 CL was dissolved in de-mineralized water and tested in concentrations of 0.01, 0.03 and 0.1%. Proparacain HCl was used as a reference compound in a concentration of 0.5% which is known to be effective in this model.

The corneal reflex test was adapted according to Régnier (1923). The conscious, trained animals were placed in a restraining cage after cutting off the lashes of both eyes. A whisker fixed to a holder was used to exert a force of approximately 150 mg. This was used to touch the cornea repeatedly in the centre of the pupil (frequency approximately 100 touches min^{-1} defined as one cycle) until the corneal reflex was elicited which was usually the case after the first or second touch under control conditions.

Two hundred μl of the test compound solution were instilled into the conjunctival sac of one eye and left in contact with the cornea for 45 s without overflow. One minute later, the second eye was tested with 200 μl of the solution containing the reference compound. Thereafter, both eyes were alternately tested for inhibition of the corneal reflex by using the whisker as described above. The number of touches necessary to elicit a corneal reflex was counted in each cycle. Complete inhibition was defined as the failure to elicit a corneal reflex after 100 touches in at least one cycle. If complete inhibition was observed, test cycles were repeated at intervals of 2 min until in two consecutive cycles a corneal reflex could be elicited with less than 100 touches. The duration of local anaesthetic action of the compound was defined as the time between the first and last cycle where complete inhibition of the corneal reflex was observed. In case no complete inhibition was obtained, the test was continued for at least 14 min. Partial inhibition of the corneal reflex was assumed if the average count of touches during all cycles of this period after drug application was higher than 3 before a corneal reflex occurred. This measure was chosen because it detects short and strong as well as weaker effects of longer duration.

Traction and maximal electroshock tests

The animals used for the maximal electroshock (MES), and traction tests were obtained from the same supplier and maintained under the same conditions as described for the AMPA-induced lethality assay.

The traction test (Boissier & Simon, 1960, modified) was performed as follows: Each animal was individually placed for accommodation for approximately 15 min in a 1l beaker. The mice were then trained to hang to a horizontal steel rod of 3 mm diameter for a period of 15 s. Thereafter, the test compound was administered to individual animals. After the respective pre-treatment time, the mouse was again held in such a position that it touched the bar with its forepaws, and was tested for its ability to hang to it for at least 15 s. If a mouse fell from the rod within 15 s, this was considered as motor impairment.

The MES test (Toman *et al*., 1946, modified) was performed as follows: Immediately following the traction test, an electroshock (20 mA/50 Hz/200 ms) was applied to the eyes *via* saline-moistened eye electrodes (Rodent shocker Type 221, HSE Electronics, March-Hugstetten, Germany). This had been determined in previous control experiments to be a supra-maximal stimulus, resulting in a fully developed tonic convulsion in 100% of the animals. Therefore, no concurrent control group was considered necessary. If the application of the electroshock after administration of the

test compound prevented the tonic convulsion, this was considered as anticonvulsive activity of the compound.

BIIR 561 CL and the reference compounds aptiganel, GYKI 52466, NBQX and mexiletine were administered either i.v. into a tail vein, or i.p., perorally (p.o.), or *via* s.c. injections 15 min prior to testing. In a subset of experiments, the traction test and the MES test were performed 15, 30, 45, 60, 90, 120, 180 and 240 min following p.o. administration.

ED_{50} values and confidence limits were calculated by a probit analysis using the SAS software program system (SAS Institute Inc., Cary, NC, U.S.A., version 6.11). A therapeutic index (TI) was defined as the ratio of the ED_{50} values for the traction test and the maximal electroshock test (ED_{50TT} : ED_{50MES}).

Amygdala kindling model

Female Wistar rats were purchased at a body weight of 200–220 g (Harlan Winkelmann Versuchstierzucht, Borchen, Germany) and were then kept under controlled conditions (24–25°C, 50–60% relative humidity, 12 h light/dark cycle) with free access to standard laboratory chow (Altromin 1324 standard diet) and tap water. All experiments were performed at the same time of day to minimize possible effects of circadian variation. During the period of experiments animals had a body weight between 270 and 425 g. These animals were previously kindled and used to test other compounds. The period between the previous and current studies was at least 1 month to provide sufficient time for a complete washout from the previously tested drug.

For implantation of kindling electrodes rats were anaesthetized with chloral hydrate (360 mg kg^{-1}, i.p.), the skull surface was exposed, and a bipolar electrode was implanted into the right hemisphere aimed at the basolateral amygdala using the following stereotaxic co-ordinates according to the atlas of Paxinos & Watson (1986): 2.2 mm caudal, 4.8 mm lateral, 8.5 mm ventral (all respective to bregma). The electrodes consisted of two twisted Teflon-coated stainless steel wires (250 μm diameter) separated by 0.5 mm at the tip. A screw, which served as grounding electrode, was positioned over the left parietal cortex. Bipolar and ground electrodes were connected to plugs, and the electrode assembly and anchor screws were held in place with dental acrylic cement applied to the exposed skull surface. After surgery, the rats were treated with antibiotics for 1 week to prevent infection.

Following a post-operative recovery period of 2 weeks, constant current stimulation (500 μA, 1 msec, monophasic square-wave pulses, 50 Hz for 1 s) were delivered to the amygdala once daily (five times per week) until at least 10 sequential fully kindled stage-5 seizures were elicited. Seizure severity (SS) was scored according to Racine (1972): 1 = immobility, eye closure, ear twitching, twitching of vibrissae, sniffing, facial clonus; 2 = head nodding associated with more severe facial clonus; 3 = clonus of one forelimb; 3.5 = bilateral clonus without rearing; 4 = bilateral clonus accompanied by rearing; 4.5 = generalized clonic seizures without rearing and falling (e.g. because of direct loss of balance); 5 = rearing and falling accompanied by generalized clonic seizures. In these fully kindled rats afterdischarge threshold (ADT) was determined by administering a series of stimulations at intervals of 1 min increasing in steps of about 20% compared to the previously applied current. The ADT was defined as the lowest current intensity producing afterdischarge with a duration of at least 5 s. Determination of ADT was repeated two times to prove reproducibility before animals were used for anticonvulsant drug testing.

In all experiments, seizure duration (SD) and afterdischarge duration (ADD) were recorded in addition to SS and ADT. SD was the time period of limbic and/or motor seizures.

ADD was defined as the period of high amplitude spiking (at least 1 Hz frequency and twice the pre-stimulation amplitude) in the electroencephalogram (EEG) of the electrode positioned in the basolateral amygdala, including the time of stimulation.

BIIR 561 CL was dissolved in 0.9% NaCl solution and administered i.p. Administered volume was 3 ml kg^{-1} bodyweight. Dosages used in the experiments were 1.1, 3.4 and 11.2 mg kg^{-1}.

Animals were allowed to adapt to the laboratory environment, then body temperature was measured and animals were put into open cages for constant observation. Fourteen min following drug or vehicle administration, behavioural alterations and body temperature were determined. Adverse effects were scored during observation in open cages and in an open field. In addition, rats were subjected to the rotarod test (polypropylene, foam-coated rod, 5 cm in diameter, 8 r.p.m.). Animals were considered to have failed this test when they fell from the rod in each of three consecutive 1 min attempts.

Drugs

BIIR 561 CL, aptiganel, GYKI 52466, and mexiletine were synthesized in the Department of Medicinal Chemistry of Boehringer Ingelheim Pharma KG. AMPA and NBQX were obtained from Tocris Cookson Ltd., U.K. Proparacain HCl was purchased from Ursapharm, Saarbrücken, Germany. All other chemicals were at least of reagent grade and purchased from reputable suppliers.

During the experimental phase, doses of BIIR 561 were calculated using the hydrochloride salt form. In this study, doses are indicated using the free base, representing the actual active chemical substance. The conversion factor of 0.8946 resulted in figures, which were rounded to one post-comma digit.

Results

Mechanism-related effects in vivo

In the first set of experiments of the series reported in this paper, BIIR 561 CL was tested for AMPA-antagonistic effects *in vivo* and local anaesthetic effects.

When administered i.v. at a dose of 60 mg kg^{-1}, AMPA induced lethality in 90–100% of the control animals within 1 min. BIIR 561 CL dose-dependently protected the animals against AMPA-induced lethality with an ED_{50} value of 4.5 mg kg^{-1} (confidence interval: 3.1–6.5 mg kg^{-1}, Figure 1a). GYKI 52466, a non-competitive AMPA antagonist, also protected against AMPA-induced lethality with an ED_{50} value of 10.1 mg kg^{-1} (confidence interval: 3.2–49.5 mg kg^{-1}, Figure 1b). For both compounds, the highest

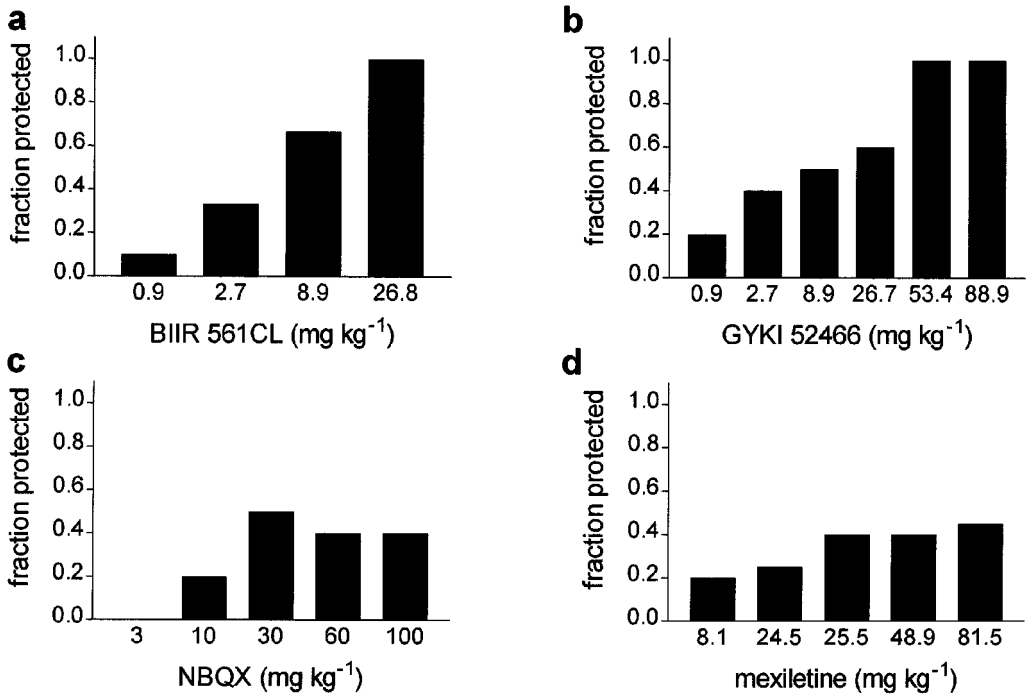

Figure 1 Effects of BIIR 561 CL and reference compounds on AMPA-induced lethality in mice. (a) BIIR 561 CL following s.c. administration, dose-dependently protected mice against an injection of AMPA, which caused death in 90–100% of the control animals (60 mg kg^{-1} i.v.) within 1 min. At the highest dose of 26.8 mg kg^{-1}, BIIR 561 CL protected all animals. The ED_{50} was 4.5 mg kg^{-1} (confidence interval: 3.1–6.5 mg kg^{-1}). Complete protection was also brought about by the non-competitive AMPA receptor blocker GYKI 52644 with an ED_{50} of 10.1 mg kg^{-1} (confidence interval: 3.2–49.5 mg kg^{-1}; b). The competitive AMPA antagonist NBQX reduced AMPA-lethality, but not more than 50% of the animals were protected even at doses of 30–100 mg kg^{-1} (c). The Na^+ channel blocker mexiletine was also protective to a limited extent (d). Aptiganel, a non-competitive blocker of NMDA-receptors, was without effect up to 8.9 mg kg^{-1} (not shown).

dose protected 100% of the animals. NBQX, a competitive AMPA antagonist, only partly protected the mice. Maximal protection of 50% was offered at 30 and 100 mg kg^{-1} (Figure 1c). Mexiletine, a Na^+ channel blocker, also provided only partial protection with a maximum effect of 30–60% in a dose range of 24.5–81.5 mg kg^{-1} s.c. (Figure 1d). Higher doses (122.3 mg kg^{-1} s.c.) induced ataxia and convulsions which appeared during the pre-treatment time. Aptiganel, a non-competitive NMDA antagonist, did not protect the animals against AMPA-induced lethality in doses up to 8.9 mg kg^{-1} that were shown to be neuroprotective in animal models of acute ischaemic stroke.

The corneal reflex test was used to test for local anaesthetic properties of BIIR 561 CL which induced a concentration-dependent inhibition of the corneal reflex (Figure 2a): At the lowest concentration of 0.01%, a complete inhibition of the corneal reflex was induced in one of four rabbits, respectively. With 0.03%, complete inhibition was observed in four of six animals. In the remaining two animals, partial inhibition was shown. The highest concentration of 0.1% induced complete inhibition of the corneal reflex in all animals tested ($n=3$).

The median duration of the complete inhibition of the corneal reflex increased with concentration (Figure 2b).

The reference compound proparacain completely inhibited the corneal reflex with a 0.5% solution in all animals tested ($n=13$) with a median duration of 26 min (range 12–72 min, Figure 2a and b).

Anticonvulsive effects

The MES test and the amygdala-kindling model were used to investigate anticonvulsive properties of BIIR 561 CL in a second set of experiments. Preliminary MES data for BIIR 561 CL have been published elsewhere (Weiser *et al*., 1999).

In the MES test, the compound induced dose- and time-dependent protection against tonic seizures after electrical stimulation when given *via* different routes of administration (Table 1).

The effect of BIIR 561 CL decreased when the time interval between drug administration and MES test was increased: Following s.c. administration, maximum protection against tonic seizures was reached with a time interval of 15 and 30 min between drug administration and MES test with an ED_{50} value of 3 mg kg^{-1} (confidence interval: 2.5–3.8 mg kg^{-1}) and an ED_{50} value of 2.8 mg kg^{-1} (confidence interval: 2.1–3.9 mg kg^{-1}), respectively. Sixty min following drug administration the ED_{50} value was 6.2 mg kg^{-1} (confidence interval: 4.8–7.8 mg kg^{-1}). At 90 min after drug administration, the ED_{50} value was 9 mg kg^{-1} (Table 1).

The effect of BIIR 561 CL varied with the route of administration: Following i.v. administration, the ED_{50} value was lower compared to s.c. and p.o. administration (1, 5.9, and 23.4 mg kg^{-1}, respectively, Table 1).

In a subset of experiments, ED_{50} values were determined following increasing pre-treatment times after p.o. administration. The ED_{50} values showed an initial decrease up to

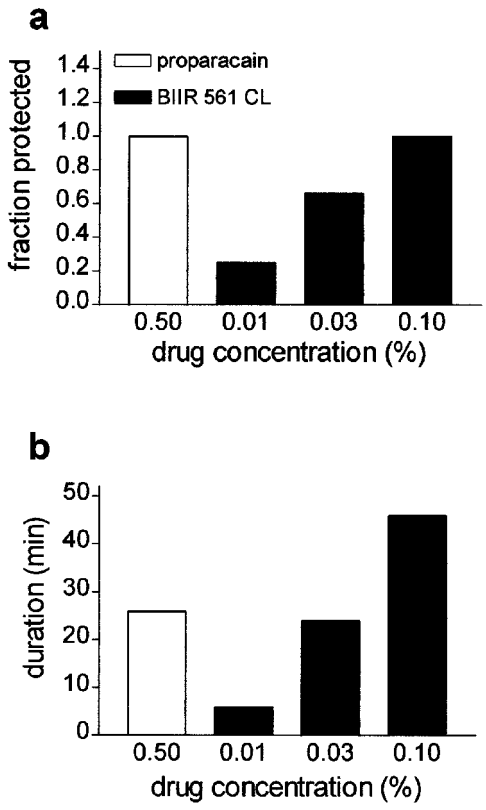

Figure 2 Effect of BIIR 561 CL and proparacain on the corneal reflex in conscious rabbits after conjunctival instillation. (a) Fraction of animals which were completely insensitive to touch of the cornea. BIIR 561 CL (filled bars) at 0.01% induced complete local anaesthesia in 25% (one out of four animals); at 0.03% 66% (four out of six animals), and at 0.1% 100% (three out of three animals) were insensitive to corneal touch. The local anaesthetic proparacain 0.5% (open bars) was completely effective in all (13 out of 13) animals at a concentration of 0.5%. (b) Duration of the effect in fully locally anaesthetized animals. At the lowest concentration of BIIR 561 CL (0.01%), the effect persisted for 6 min. At higher doses, the median duration of full local anaesthesia was increased to 24 min (range: 14–30 min) at 0.03%, and 46 min (range: 46–54 min) at 0.1%, respectively. Proparacain at 0.5% was fully effective for 26 min (range: 12–72 min).

60 min pre-treatment time followed by an increase for 90, 120 and 180 min pre-treatment times (Table 1).

The reference compounds aptiganel, GYKI 52466, NBQX and mexiletine also provided protection against seizures following electrical stimulation measured 15 min after s.c. administration with ED_{50} values of 2.6 mg kg^{-1} (aptiganel), 6.9 mg kg^{-1} (GYKI 52466), 36 mg kg^{-1} (NBQX), and 2 mg kg^{-1} (mexiletine) (Table 1).

In order to quantify the difference between doses producing protection against seizures and side effects such as motor impairment, the TI was calculated. For BIIR 561 CL, the TI was independent of the route of administration and the pre-treatment times. BIIR 561 CL induced motor impairment at doses higher than those providing protection in the MES test resulting in a TI of 9–11 (Table 1). In contrast, aptiganel had a TI < 1, indicative of motor impairment occurring at doses lower than those providing protection in the MES test. The AMPA antagonists NBQX and GYKI 52466 had TIs of 2.4 and 2.1, respectively.

BIIR 561 CL showed an anticonvulsant effect in amygdala-kindled rats (Figure 3). With the different doses an average increase in ADT of 52% (1.1 mg kg^{-1}), 44% (3.3 mg kg^{-1}) and 120% (11.2 mg kg^{-1}) above control threshold was found. This increase proved to be significant following a dose of 11.2 mg kg^{-1}. (Figure 3a). With lower doses there were individual differences with only four of nine (1.1 mg kg^{-1}) or five of ten (3.4 mg kg^{-1}) animals responding with an ADT increase of at least two steps above control.

Seizure severity was almost not influenced by 1.1 mg kg^{-1} BIIR 561 CL, but was reduced to 78% of control with higher doses. This reduction reached statistical significance for the experiment with 11.2 mg kg^{-1} BIIR 561 CL (Figure 3b).

Seizure duration was reduced in a dose-dependent manner with significant reduction following the doses of 3.4 and 11.2 mg kg^{-1}. Thereby SD was reduced to 56 and 45% (Figure 3c). Furthermore, BIIR 561 CL decreased ADD significantly to 56% at 11.2 mg kg^{-1} (Figure 3d).

With all three doses, no signs of behavioural adverse effects such as ataxia, loss of righting reflex, sedation or locomotor impairment during observation and rotarod test were found.

Discussion and conclusions

BIIR 561 CL has recently been described as a novel compound combining AMPA receptor- and Na^+-channel blocking properties (Weiser *et al.*, 1999). This combination may offer protection in diseases of the CNS such as epilepsy or acute neurodegeneration as a consequence of ischaemic stroke where both, AMPA antagonists as well as Na^+ channel blockers have been reported to be active (Gill, 1994; Lees, 1996; Taylor & Narasimhan, 1997). However, a combined block of currents through AMPA receptor-related channels and voltage-dependent Na^+ channels may give rise to heightened adverse side effects which have also been reported for these compound classes (Lees, 1996; Taylor & Narasimhan, 1997). Thus, the present series of studies was undertaken in order to characterize the pharmacological effects of BIIR 561 CL in animal models related to its dual mechanisms of action.

The effects of BIIR 561 CL were compared to those of the non-competitive AMPA antagonist GYKI 52466, a 2,3-benzodiazepine which has anticonvulsant properties (Löscher & Hönack, 1994), NBQX, a competitive AMPA antagonist which was discontinued from development in the indication acute ischaemic stroke (Pharmaprojects, 1998) due to problems arising from the low solubility of the compound, and mexiletine, an anti-arrhythmic drug and Na^+ channel blocker which was also described as anticonvulsive (Alexander *et al.*, 1986).

Neuronal damage occurring during conditions such as acute ischaemic stroke or brain injury are associated with excessive release of excitatory amino acids and a resulting over-stimulation of glutamate receptors (Benveniste *et al.*, 1984; Faden *et al.*, 1984; Doble, 1995). This pathophysiological condition can be effectively mimicked by infusion of AMPA, which results in death due to excitotoxicity. Consequently, antagonists blocking AMPA receptors should provide protection against AMPA-induced lethality. The present data show that both non-competitive AMPA antagonists, BIIR 561 CL and GYKI 52466, provide full

Table 1 The anticonvulsive effect of BIIR 561 CL was investigated using the maximal electroshock (MES) test in mice. Various pre-administration intervals and routes of administration were tested. Impairment of motor co-ordination was assessed using the traction test.

t of preadministration (min)	*Route*	*MES* ED_{50} (mg kg^{-1})	*Traction test* ED_{50} (mg kg^{-1})	*TI*
BIIR 561 CL				
15	s.c.	3.0 (2.5–3.8)	34.4 (26.3–46.7)	11.5
30	s.c.	2.8 (2.1–3.9)	>8.9	
60	s.c.	6.2 (4.8–7.8)	>8.9	
90	s.c.	9.0	>8.9	
15	i.p.	5.9 (4.4–7.4)	>8.9	
15	i.v.	1.0 (n.d.)	>8.9	
15	p.o.	23.4 (13.9–34.2)	>89.5	
30	p.o.	23.4 (17.5–30.0)	>30	
45	p.o.	15.7 (9.3–28.0)	>30	
60	p.o.	10.8 (n.d.)	>30	
90	p.o.	30.0 (n.d.)	>100	
120	p.o.	21.2 (11.8–36.6)	>100	
180	p.o.	30.0 (n.d.)	296 (n.d.)	9.9
GYKI 52466				
15	s.c.	6.9 (5.0–10.0)	14.1 (10.0–24.2)#	2.1
Mexiletine				
15	s.c.	2.5 (n.d.)	–#	
NBQX				
15	s.c.	36.1 (19.8–65.5)	87.0 (42.8–>100)	2.4
Aptiganel				
15	s.c.	2.6 (n.d.)	2.4 (n.d.)	0.9

Data are shown as means with confidence limits in brackets (n.d.: not defined). ED_{50TT}, ED_{50MES}: ED_{50} values for the traction test (TT), and the MES test, respectively. The therapeutic index (TI) was defined as ED_{50TT} : ED_{50MES}. #: data from Weiser *et al.* (1999).

protection of mice in this model, the latter, however, being only about half as potent as BIIR 561 CL. In contrast, NBQX, a competitive AMPA antagonist, provided only partial protection and higher doses tended to be even less protective. Similar results were reported by Yamaguchi *et al.* (1993) who showed that GYKI 52466 but not NBQX fully protected against AMPA-induced toxicity. Interestingly, mexiletine, a blocker of voltage-dependent Na^+ channels, also provided some protection against AMPA-induced lethality which may be explained by a mexiletine-induced decrease of pre-synaptically released glutamate in response to AMPA-induced neuronal depolarization. This is supported by reports where mexiletine was shown to counteract neuronal over-stimulation in different seizure models in rodents (Alexander *et al.*, 1986). The Na^+ channel blockers BW1003C87 and lamotrigine have also been shown to pre-synaptically inhibit glutamate release (Gaspary *et al.*, 1994; Klamt, 1998). Weiser *et al.* (1999) described the Na^+ channel blocking properties of BIIR 561 CL in various *in vitro* models. The Na^+ channel blocker mexiletine was also active in these models albeit with a factor between 10 and 17 less potent than BIIR 561 CL.

BIIR 561 CL had local anaesthetic properties in the corneal reflex model which was first described by Régnier (1923), and has since then been an established test for characterization of local anaesthetic agents. A 0.1% solution of BIIR 561 CL was equally effective as a 0.5% solution of the local anaesthetic proparacain, and the anaesthesia provided by BIIR 561 CL lasted approximately twice as long as that induced by proparacain in these concentrations. The similarity of the effects induced by both drugs suggests that the local anaesthesia mediated by BIIR 561 CL may reflect it's Na^+ channel blocking activity.

Phenytoin and carbamazepine are anti-epileptic drugs which block Na^+ channels, and both, Na^+ channel blockers as well as AMPA receptor antagonists, have been repeatedly reported to have anticonvulsant activity (Taylor & Narasimhan, 1997; Löscher, 1998). Therefore, in the present study it was investigated whether BIIR 561 CL was active in different models of epilepsy with convulsions being induced by amygdala kindling as a model for temporal lobe epilepsy, or electrically (MES test), where BIIR 561 CL was tested using different routes of administration and different pre-treatment times.

The effects of BIIR 561 CL in the amygdala kindling model are comparable to those found with standard anti-convulsants (Löscher *et al.*, 1993a). Other AMPA receptor antagonists such as NBQX or GYKI 52466 were also active in the kindling model of epilepsy (Löscher, 1998). NBQX reduced focal seizure threshold and seizure severity when administered with 30 mg kg^{-1} (Löscher *et al.*, 1993b) which is in good agreement with the doses needed to provide protection in the AMPA-induced lethality model and the MES test reported here.

With regard to the good predictability of the kindling model for drug efficacy against complex-partial seizures in humans (Sato *et al.*, 1990), the present data may predict an anticonvulsant activity of BIIR 561 CL in temporal lobe

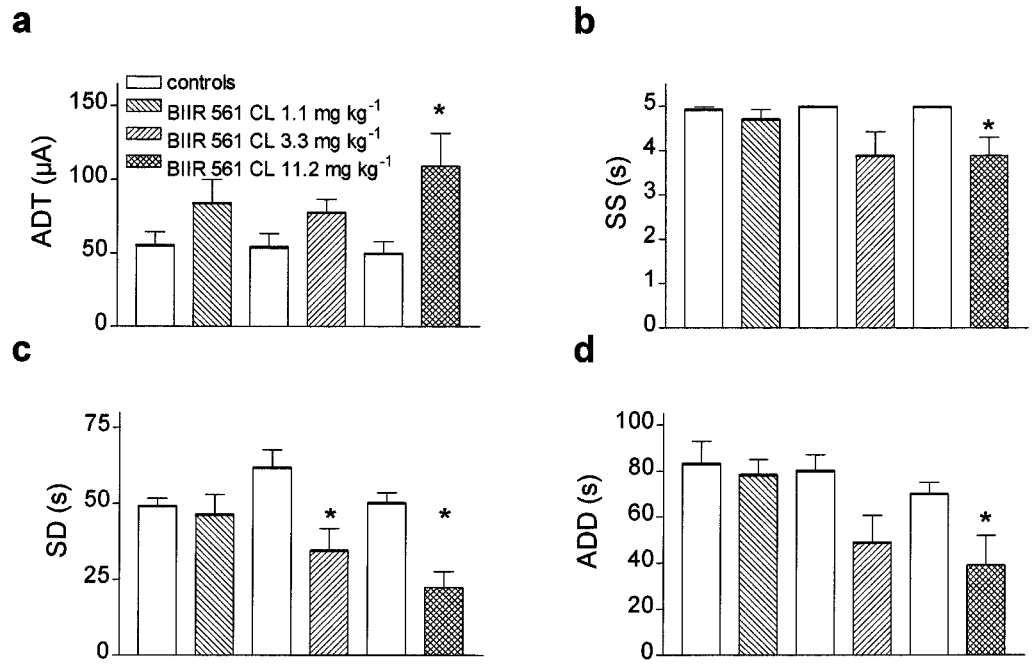

Figure 3 Effects of BIIR 561 CL on amygdala-kindled rats. (a) The afterdischarge threshold (ADT) was determined by administering current stimulation of increasing intensities in fully kindled animals. BIIR 561 CL (i.p.) at all doses tested increased the ADT; at 11.2 mg kg^{-1} ADT was increased by 120% above control. (b) The severity of seizures (SS) was reduced by 11.2 mg kg^{-1} to 78%. (c) Seizure duration (SD) was reduced to 56% (for 3.4 mg kg^{-1}), and 45% (11.2 mg kg^{-1}), respectively. (d) The duration of afterdischarges (ADD) was reduced to 56% by the highest dose of BIIR 561 CL. Statistical significance was calculated by Wilcoxon signed rank test for paired replicates. Significant differences to the controls (C, $P<0.05$) are marked by asterisks. Data are means plus s.e. of a group of nine fully kindled rats. Vehicle control data (open bars) were recorded 2–3 days before the drug trial (hatched bars) for each dose.

epilepsy. Furthermore, the compound did not induce behavioural adverse effects at the doses tested, and thus seems to have a favourable side effect profile.

The question whether the anticonvulsant activity of BIIR 561 CL may be attributed more to its AMPA antagonistic– or Na^+ channel blocking properties is difficult to address. However, compounds from the series of molecules from which BIIR 561 CL emerged that had comparable AMPA antagonistic properties as BIIR 561 CL but were less active in blocking Na^+ channels, where less efficacious in the MES test than BIIR 561 CL (unpublished observation). This suggests that both modes of action may contribute the the anticonvulsant activity of BIIR 561 CL.

In the MES test, the ED_{50} values increased from i.v. to s.c. to p.o. administration which may be explained by pharmacokinetic factors such as lower bioavailability after s.c. and p.o. administration compared to the i.v. route. When the pre-treatment time was increased from 15 to 90 min, the ED_{50} value increased also which indicates metabolism and/or excretion. Following p.o. administration, the ED_{50} value did not change much even up to 180 min pre-treatment time, which could indicate the occurrence of an active metabolite when given *via* the oral route. Interestingly, the TI was affected neither by the route of administration nor by the duration of the pre-treatment time. BIIR 561 CL had a TI of about 11 compared to 2 for GYKI 52466, 2.4 for NBQX and 0.9 for aptiganel, a non-competitive NMDA receptor antagonist. The better separation between protection against convulsions and induction of motor impairment seen with BIIR 561 may be explained by the fact that this compound has a dual mechanism of action which may act synergistically regarding suppression of seizures. These findings are supported by Yamaguchi *et al.* (1993) who also reported poor separation between protection against MES seizures and induction of motor toxicity for GYKI 52466 and NBQX. These authors found a similar potency for GYKI 52466 in the MES test as reported here (ED_{50} values 11.8 mg kg^{-1} *versus* 6.9 mg kg^{-1} s.c.).

In conclusion, the present study provides evidence that BIIR 561 CL has robust anticonvulsant effects in different models, exerts local anaesthetic effects and offers protection in a model mimicking excessive glutamate release as is encountered in conditions of neurotrauma and acute ischaemic stroke. These protective effects make BIIR 561 CL an attractive candidate for neuroprotective therapy of diseases of the central nervous system such as epilepsy and stroke.

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