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Anticonvulsant profile of the imidazoquinazolines NNC 14-0185 and NNC 14-0189 in rats and mice

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Abstract

The anticonvulsant effects of NNC 14-0185 (3-(3-cyclopropyl-5-isoxazolyl)-6-fluoro-5-morpholino-imidazo[1,5-*a*]quinazoline) and NNC 14-0189 (3-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)-7-fluoro-5-(4-methyl-1-piperazinyl)-imidazo[1,5-*a*]quinazoline) in mice and rats were evaluated and compared with those of diazepam, clonazepam and the novel β-carboline, abecarnil. Following i.p. administration, NNC 14-0185 and NNC 14-0189 prevented audiogenic seizures in DBA/2 mice and the clonic convulsions induced in mice by pentylenetetrazole, DMCM (methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate), 3-mercaptopropionic acid and a low dose of bicuculline. NNC 14-0185 and NNC 14-0189 prevented seizures induced by pentylenetetrazole in rats and were also effective anticonvulsants in amygdala-kindled rats. In general, the anticonvulsant potencies of NNC 14-0185 and NNC 14-0189 were comparable to those of the reference benzodiazepines. However, like abecarnil, they were not effective against the seizures induced in mice by maximal electroshock and a high dose of bicuculline. The anticonvulsant effects of NNC 14-0185 and NNC 14-0189 against pentylenetetrazole-induced seizures were apparent within 5 min of i.p. injection and persisted for at least 2 h. These effects appeared to be mediated by benzodiazepine receptors since they were inhibited by concurrent administration of flumazenil. Both NNC 14-0185 and NNC 14-0189 showed greater separation between their anticonvulsant and muscle relaxant effects (measured as impaired rotarod performance) than did diazepam. In this respect, their therapeutic windows were similar (NNC 14-0185) to or better (NNC 14-0189) than that of abecarnil. Tolerance did not develop to the anticonvulsant effects of NNC 14-0185 and NNC 14-0189 over a 4-day test. In comparison, the anticonvulsant effects of diazepam and abecarnil were attenuated by repeated drug administration. Thus, NNC 14-0185 and NNC 14-0189 have a promising anticonvulsant and side-effect profile in comparison with diazepam, clonazepam and abecarnil. The potential use of these compounds in the treatment of epilepsy should be explored further.

Keywords: NNC 14-0185; NNC 14-0189; Benzodiazepine receptor; Seizure threshold; Epilepsy

1. Introduction

Benzodiazepine receptor agonists have been shown to be efficacious anticonvulsant agents in experimental animals and in humans. The advantages of benzodiazepine agonists as antiepileptic drugs are that they have a rapid onset of action, a broad spectrum of activity and relatively low toxicity. However, the use of benzodiazepines in the treatment of epilepsy is restricted by the development of rapid tolerance to their anticonvulsant action and a high degree of muscle relaxant, sedative and amnesic side effects (Schmidt et al., 1986; Haigh and Feely, 1988; Rogawski and Porter, 1990). This has led to a search for benzodiazepine receptor agonists with reduced adverse effects.

NNC 14-0185 and NNC 14-0189 are novel imidazo[1,5-a]quinazolines (Fig. 1) which were synthesized in the Medicinal Chemistry Department at Novo Nordisk. NNC 14-0185 and NNC 14-0189 potently displace [^{3}H]flunitrazepam from its binding sites in bovine cortex membrane preparations (IC_{50} 5.6 and 7.9 nM, respectively, compared with 11 nM for diazepam), however, they have negligible affinity for a wide variety of other receptor and uptake sites in mammalian brain including those asso-

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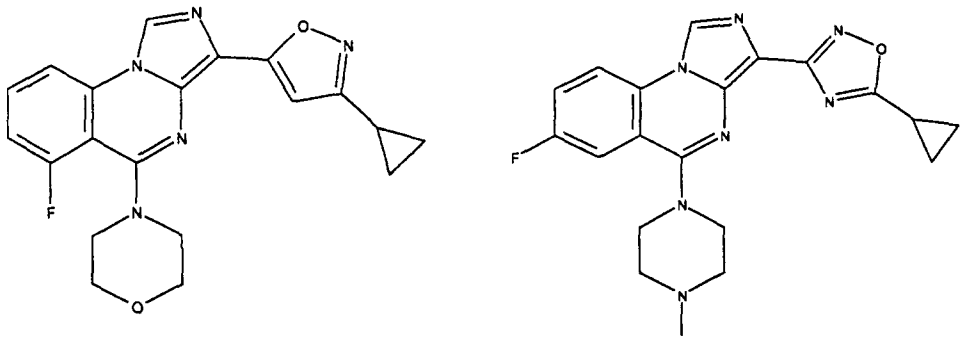

NNC 14-0185 NNC 14-0189

Fig. 1. The structures of NNC 14-0185 and NNC 14-0189.

ciated with epilepsy, such as GABA (γ-aminobutyric acid) receptors and uptake sites, purine receptors, excitatory amino acid receptors and calcium ion channels (P. Suzdak, unpublished observations). Benzodiazepine receptor agonists potentiate $GABA_A$ receptor function. Therefore the anticonvulsant effects of NNC 14-0185 and NNC 14-0189 were evaluated against chemical convulsants which interfere with GABAergic function (see review by De Deyn et al., 1992) including pentylenetetrazole which interacts with the $GABA_A$ receptor complex, possibly at the picrotoxin site (Macdonald and Barker, 1978; Olsen, 1981), the $GABA_A$ receptor antagonist, bicuculline, the benzodiazepine receptor inverse agonist, DMCM (methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate) and the glutamate decarboxylase inhibitor, 3-mercaptopropionic acid. The effects of the imidazoquinazolines against electrically induced seizures in rats and mice and in an animal model of reflex epilepsy were also examined. Finally, the ability of NNC 14-0185 and NNC 14-0189 to produce muscle relaxation was assessed in the rotarod test, and the potential development of tolerance to their anticonvulsant action was investigated following repeated drug administration.

The anticonvulsant and side-effect profiles of NNC 14-0185 and NNC 14-0189 in the different animal models were compared with those of diazepam, clonazepam and abecarnil. Clonazepam is currently available for the treatment of epilepsy and abecarnil is a novel β-carboline which has been shown to have a promising anticonvulsant profile in rats, mice and primates with good separation between its ability to prevent seizures and its muscle relaxant effects (Turski et al., 1990). Some of the results presented here have been published in abstract form (Jackson et al., 1995).

2. Materials and methods

2.1. Animals

Experiments were performed on male NMRI mice; male Wistar rats and male DBA/2 mice. Mice were obtained from Bomholdtgård, Ry (Denmark) and rats were obtained from Møllegård, Lille Skensved (Denmark). The body weights of the animals are given in the appropriate sections below. All animals were housed in conventional group cages before experimentation, with free access to a standard rat and mouse diet and tap water. Animal rooms were maintained at a constant temperature (21–23°C) and relative humidity (40–60%) and on a 12 h light-dark cycle (lights on at 06:00 h). All experiments were performed between 08:00 h and 15:00 h.

2.2. Sound-induced seizures in mice

Antagonism of sound-induced seizures was investigated in male DBA/2 mice (7–9 g). Groups of ten mice were injected i.p. with vehicle or one of at least four different doses of the test drug. Thirty minutes later the mice were placed in pairs in the test chamber (25 × 22 × 15 cm) and exposed to a 111 dB 14 kHz sinus tone for 30 s through a speaker in the ceiling. The animals were observed for the occurrence of clonic convulsions during exposure to the sound.

2.3. Chemically induced seizures in mice

Male NMRI mice (18–22 g; $n = 7–8$) were injected i.p. with vehicle or one of at least four different doses of the test drug 30 min before administration of pentylenetetrazole (160 mg/kg s.c.); bicuculline (2.7 or 5.0 mg/kg s.c.); DMCM (18 mg/kg i.p.) or 3-mercaptopropionic acid (60 mg/kg i.p.). The animals were observed over the subsequent 15 (pentylenetetrazole)- or 30 (bicuculline, DMCM and 3-mercaptopropionic acid)-min period for the presence of clonic seizures. The dose of chemical convulsant used in this study (and the duration of the observation period for seizure activity) was determined following dose-response studies in NMRI mice in our laboratory.

A separate set of experiments served to evaluate the anticonvulsant effects of the benzodiazepine receptor agonists in the sensitive pentylenetetrazole infusion test. Male NMRI mice (18–22 g; $n = 10$) were injected with vehicle

or a number of different doses of the test drug. Thirty minutes later, the mice were given an i.v. infusion of pentylenetetrazole (15 mg/ml; 12 ml/h) through the tail vein. The amount of pentylenetetrazole required to produce clonic seizures (mg/kg pentylenetetrazole i.v.) was determined for each animal. The results are expressed as treatment group means ± S.E.M.

2.4. Maximal electroshock in mice

Male NMRI mice (20–25 g; $n = 7–8$) were injected i.p. with vehicle or one of at least four different doses of the test drug and exposed 30 min later to a 60-Hz alternating current of 50 mA for 0.2 s. The current was applied via corneal electrodes using a HSE-shock Reizgerät, type 207, obtained from Hugo Sachs Electronic (Germany). The mice were observed for the presence of tonic hindlimb extension for the next 30 s.

2.5. Time course of anticonvulsant action in mice

The time course of the anticonvulsant action of NNC 14-0185 and NNC 14-0189 was assessed in male NMRI mice (18–22 g; $n = 6–8$) using the pentylenetetrazole infusion test described in Section 2.3. The amount of pentylenetetrazole required to produce clonic seizures in each animal (mg/kg pentylenetetrazole i.v.) was calculated at different times (5, 15, 30, 60 min; 2, 4 and 6 h) following administration of NNC 14-0185 (10 mg/kg i.p.) or NNC 14-0189 (10 mg/kg i.p.). The dose of NNC 14-0185 and NNC 14-0189 used in the time course studies was chosen from the dose-response curves shown in the Results section. The results of the time course experiment are expressed as treatment group means (mg/kg pentylenetetrazole i.v.) ± S.E.M.

2.6. Flumazenil reversibility studies in mice

The pentylenetetrazole infusion test was used to investigate whether the anticonvulsant effects of NNC 14-0185 and NNC 14-0189 could be attenuated by the benzodiazepine receptor antagonist, flumazenil. Male NMRI mice (18–22 g; $n = 8$) were treated concurrently with either vehicle or the test drug (10 mg/kg i.p.) and either vehicle or flumazenil (10 mg/kg i.p.). Fifteen minutes later pentylenetetrazole was infused via the tail vein as described above (Section 2.3) and the amount of pentylenetetrazole (mg/kg i.v.) required to produce clonic seizures was calculated for each mouse. The dose of flumazenil employed in this study was chosen from the literature (e.g. see Nutt et al., 1982) and is routinely used to block the behavioural effects of benzodiazepine receptor agonists in rats and mice. The dose of NNC 14-0185 and NNC 14-0189 used was chosen from the dose-response curves shown in the Results section. The results of the flumazenil reversibility experiments are expressed as treatment group means (mg/kg pentylenetetrazole i.v.) ± S.E.M.

2.7. Antagonism of pentylenetetrazole-induced seizures in rats

Male Wistar rats (110–130 g; $n = 8$) were injected i.p. with vehicle or one of at least four different doses of the test drug. Thirty minutes later the animals were given pentylenetetrazole (170 mg/kg s.c.) and observed for the subsequent 30 min for the presence of clonic seizures. This dose of pentylenetetrazole was chosen following pilot studies in our laboratory.

2.8. Antagonism of electrically induced seizures in amygdala-kindled rats

Male Wistar rats (180–220 g) were anaesthetised with pentobarbital and implanted with bipolar electrodes in the right amygdaloid nucleus. Kindled seizures were induced by applying a constant current stimulation (300 μA, 1 ms, biphasic square wave pulses with a frequency of 50 Hz, for 1 s) to the amygdala three times a week. Rats were considered to be fully kindled when they exhibited three consecutive generalised clonic convulsions associated with loss of balance (stage 5 seizures; Racine, 1972) in response to the electrical stimulation. In these experiments, each animal acted as its own control i.e., kindled seizures were evaluated 30 min after i.p. administration of vehicle and, two days later, 30 min after i.p. administration of one of four different doses of the test drug to the same animals. Treatment groups contained 5–7 rats which were in the weight range 450–550 g at the time of the experiment. The animals were observed for the presence of clonic seizures (i.e. myoclonic jerks, clonic convulsions in the forelimbs or generalised convulsions with loss of balance i.e., stage 3 to 5 seizures according to the Racine scale; Racine, 1972) in response to electrical stimulation of the amygdala.

2.9. Tolerance studies in mice

The pentylenetetrazole infusion test was used to assess changes in seizure threshold following repeated administration of NNC 14-0185 (10 mg/kg i.p.); NNC 14-0189 (10 mg/kg i.p.); diazepam (10 mg/kg i.p.); clonazepam (0.3 mg/kg i.p.) and abecarnil (3 mg/kg i.p.). These doses of the benzodiazepine receptor agonists were chosen as they all produced an increase in pentylenetetrazole seizure threshold of about 200 mg/kg i.v. as shown in Results. Male NMRI mice (18–22 g at the start of the experiment; $n = 10$) were injected i.p. twice a day (08:00 h and 16:00 h) for three days with the test drug. Clonic pentylenetetrazole seizure thresholds were measured 30 min after drug treatment on day 4. Acute drug effects were measured in mice injected with vehicle twice a day for

three days and given the test compound on day 4. Control animals were injected with vehicle throughout the experiment. The results are expressed as treatment group means (mg/kg pentylenetetrazole i.v.) ± S.E.M.

2.10. Measurement of rotarod performance in mice and rats

Rotarod performance was assessed during pentylenetetrazole-seizure testing (rats and mice; $n = 7–8$) and in amygdala-kindled rats ($n = 5–7$). All animals were pretrained in the rotarod apparatus (Ugo-Basile, Italy) for 2 min, 2–3 h before the experiment began. The effects of the test drugs on rotarod performance were examined in the 5 min before seizure testing i.e., 25–30 min following i.p. drug administration. Rotarod performance was defined as impaired if the animal fell more than twice from the rotating rod (speed, 6 r.p.m.; 3 cm diameter, mice; 6 cm diameter, rats) in the 2-min test period.

2.11. Drugs

Diazepam, clonazepam and flumazenil were gifts from Hoffman-La Roche (Basle). NNC 14-0185 (3-(3-cyclopropyl-5-isoxazolyl)-6-fluoro-5-morpholino-imidazo-[1,5-*a*]quinazoline); NNC 14-0189 (3-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)-7-fluoro-5-(4-methyl-1-piperazinyl)-imidazo[1,5-*a*]quinazoline) and abecarnil were synthesized in the Medicinal Chemistry Department at Novo Nordisk A/S. All test drugs were ultrasonically suspended in water containing 5% chremophore. Pentylenetetrazole (Nordisk Droge, Copenhagen) and 3-mercaptopropionic acid (Sigma, St Louis, MI, USA) were dissolved in 0.9% saline. DMCM (gift from Schering AG, Berlin) was dissolved in 0.02 N HCl adjusted with NaOH and bicuculline (Sigma) was dissolved in 3.5% dimethylsulphoxide (DMSO). Drugs were administered in an injection volume of 10 ml/kg (NMRI mice); 12.5 ml/kg (DBA/2 mice) or 8 ml/kg (rats).

2.12. Statistical analysis

In studies applying the pentylenetetrazole infusion test, the significance of differences between treatment group mean clonic seizure thresholds (mg/kg pentylenetetrazole i.v.) was determined using an analysis of variance followed by Dunnett's test. In all other experiments, computer-programmed log-probit methods were used to calculate ED_{50} values (i.e. the dose of drug preventing seizures or impairing rotarod performance in 50% of the animals) and their associated 95% confidence limits.

3. Results

NNC 14-0185 and NNC 14-0189 potently protected against audiogenic seizures in DBA/2 mice as shown in Table 1. The ED_{50} values for the anticonvulsant effects of NNC 14-0185 and NNC 14-0189 in this model were similar to those for diazepam, clonazepam and abecarnil.

The two imidazoquinazolines also prevented the seizures induced in mice by pentylenetetrazole, a low dose of the $GABA_A$ receptor antagonist, bicuculline, the benzodiazepine receptor inverse agonist, DMCM and the glutamate decarboxylase inhibitor, 3-mercaptopropionic acid (Table 1 and Fig. 2). NNC 14-0185 and NNC 14-0189 had a potency similar to that of diazepam against the seizures induced by pentylenetetrazole although they were less potent than clonazepam (Table 1 and Fig. 2). NNC 14-0185 and NNC 14-0189 were less potent than the reference benzodiazepine receptor agonists against the clonic convulsions induced by bicuculline (2.7 mg/kg s.c.) and, like abecarnil, were inactive against the seizures produced by a higher dose of bicuculline (5 mg/kg s.c.). Diazepam and clonazepam prevented bicuculline-induced seizures, but only at relatively high doses. NNC 14-0185 and NNC 14-0189 were generally equipotent with the reference benzodiazepine receptor agonists against the clonic convulsions induced by DMCM and 3-mercaptopropionic acid,

Table 1
Anticonvulsant and muscle relaxant (rotarod performance) effects of NNC 14-0185, NNC 14-0189 and reference benzodiazepine receptor agonists in mice

Test	NNC 14-0185	NNC 14-0189	Diazepam	Clonazepam	Abecarnil
Sound-induced seizures	0.02 (0.01–0.05)	0.09 (0.04–0.18)	0.1 (0.04–0.18)	0.008 (0.003–0.03)	0.01 (0.004–0.03)
Pentylenetetrazole 160 mg/kg s.c.	3.6 (2.0–6.4)	3.1 (1.4–6.7)	0.8 (0.6–1.2)	0.1 (0.05–0.3)	0.5 (0.3–1.2)
Bicuculline 2.7 mg/kg s.c.	4.3 (0.3–13.9)	11.8 (4.8–29.8)	0.2 (0.1–0.4)	0.04 (0.02–0.09)	0.4 (0.2–0.8)
Bicuculline 5 mg/kg s.c.	> 100	> 100	3.6 (2.2–8.2)	0.6 (0.2–1.4)	> 100
DMCM 18 mg/kg i.p.	0.4 (0.04–1.5)	0.8 (0.2–2.7)	1.2 (0.6–2.0)	0.1 (0.05–0.3)	0.007 (0.002–0.01)
3-Mercaptopropionic acid 60 mg/kg i.p.	0.8 (0.4–2.8)	2.8 (1.5–6.6)	0.9 (0.4–1.8)	0.2 (0.03–1.2)	0.6 (0.3–1.1)
Maximal electroshock	> 30	> 30	3.8 [a]	> 30	> 30
Rotarod performance	35 (11–1631) [b]	56 (31–135)	1.3 (0.5–4.5)	0.4 (0.2–0.8)	5.7 (1.5–18.4)

Results are ED_{50} values (mg/kg i.p.; 30-min pretreatment) and 95% confidence limits. At least 4 doses of each drug were administered. $n = 7–10$ (as described in Methods). [a] 95% confidence limits could not be calculated due to the steep dose-response curve (1/7 mice were protected at 3 mg/kg and 7/7 mice at 10 mg/kg). [b] 5/7 mice impaired at 100 mg/kg.

Table 2
Therapeutic indices. Ratios of ED_{50} values for inhibition of rotarod performance and protection against pentylenetetrazole-induced clonic seizures in mice and rats and inhibition of rotarod performance and protection against electrically induced seizures in amygdala-kindled rats

Compound	Mice	Rats	Amygdala-kindled rats
NNC 14-0185	10	4.5	2.5
NNC 14-0189	18	10	3.6
Diazepam	1.6	1	1.2
Clonazepam	4	1	Not tested
Abecarnil	11	5	0.8

The therapeutic indices were calculated using the results shown in Table 1 and Figs. 5 and 6. Rotarod performance was measured a few minutes before seizure testing i.e. anticonvulsant and ataxic effects were assessed in the same animals.

the notable exception being abecarnil, which prevented DMCM-induced seizures at very low doses.

Diazepam was active in the mouse maximal electroshock test (Table 1), however, NNC 14-0185, NNC 14-0189, clonazepam and abecarnil were not anticonvulsant against electrically induced seizures in mice in doses up to 30 mg/kg i.p.

The effects of the five benzodiazepine receptor agonists on rotarod performance in mice are shown in Table 1. Therapeutic indices are shown in Table 2. NNC 14-0185 and NNC 14-0189 did not impair rotarod performance except at very high doses i.e., there was a 10- and 18-fold separation between the ability of these compounds to prevent pentylenetetrazole-induced seizures in mice and to impair rotarod performance (Table 2). A similar separation was observed for abecarnil. The therapeutic indices of NNC 14-0185, NNC 14-0189 and abecarnil were much greater than those of diazepam and clonazepam, which produced ataxia at doses only slightly greater than those preventing pentylenetetrazole-induced seizures.

The time course of the anticonvulsant action of NNC 14-0185 and NNC 14-0189 is shown in Fig. 3. Both NNC 14-0185 (10 mg/kg i.p.) and NNC 14-0189 (10 mg/kg i.p.) increased clonic pentylenetetrazole seizure thresholds to over 250 mg/kg i.v. in the first 5 min after injection. The anticonvulsant effects of NNC 14-0185 were maintained for 2 h. At the 4-h reading, clonic seizure thresholds of mice given NNC 14-0185 were similar to those of the vehicle-treated controls. NNC 14-0189 appeared to be slightly longer-acting than NNC 14-0185 as the seizure thresholds of mice treated with NNC 14-0189 did not return to their control levels until 6 h after injection. The time courses of the anticonvulsant action of diazepam (2 mg/kg i.p.) and abecarnil (3 mg/kg i.p) were similar to those for NNC 14-0185 and NNC 14-0189 i.e., the increase in seizure threshold produced by the compounds was most evident in the first 2 h after injection (data not shown).

The benzodiazepine receptor antagonist, flumazenil (10 mg/kg i.p.), significantly antagonised the increase in pentylenetetrazole seizure threshold produced by NNC 14-0185 (10 mg/kg i.p.) as shown in Fig. 4. The seizure thresholds of mice treated with NNC 14-0185 and flumazenil were still significantly greater than those of the vehicle-treated control group. The increase in seizure threshold induced by NNC 14-0189 (10 mg/kg i.p.) was completely inhibited by flumazenil. This dose of flumazenil completely antagonised the anticonvulsant effects of diazepam (10 mg/kg i.p.) and abecarnil (3 mg/kg i.p.) under these conditions (data not shown). Flumazenil (10

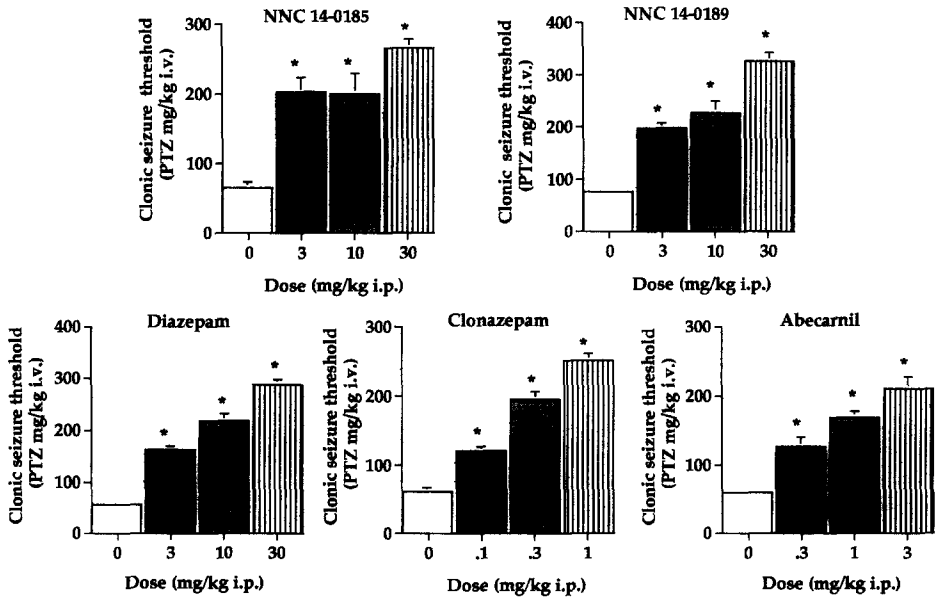

Fig. 2. Anticonvulsant effects of NNC 14-0185, NNC 14-0189 and reference benzodiazepine receptor agonists in the pentylenetetrazole (PTZ) infusion test in mice. The results are expressed as means and S.E.M. for groups of 10 mice. Significant differences from the vehicle-treated group are shown by * $P < 0.05$.

mg/kg i.p.) had no effect on the pentylenetetrazole clonic seizure threshold when given alone (Fig. 4).

NNC 14-0185 and NNC 14-0189 inhibited pentylenetetrazole-induced clonic seizures in rats in a dose-dependent manner (Fig. 5). The ED_{50} values of NNC 14-0185 and NNC 14-0189 for protection against pentylenetetrazole-induced convulsions in rats were 1.3 and 4.1 mg/kg i.p. The anticonvulsant effects of NNC 14-0185 and NNC 14-0189 in this model were similar to those of diazepam, clonazepam and abecarnil (ED_{50} values of 3.5, 1.1 and 0.4 mg/kg i.p. respectively). A clear separation was observed between the anticonvulsant and the ataxic effects of NNC 14-0185 and NNC 14-0189 as shown in Fig. 5. The doses of NNC 14-0185 and NNC 14-0189 required to impair rotarod performance in 50% of the rats were 5.9 and 40 mg/kg i.p. compared with 3.5, 1 and 2 mg/kg i.p. for diazepam, clonazepam and abecarnil. The therapeutic indices (ratio of ED_{50} values for inhibition of rotarod performance and protection against pentylenetetrazole-induced clonic seizures) of the benzodiazepine receptor agonists in rats are shown in Table 2. The therapeutic windows of NNC 14-0185 and NNC 14-0189 in these animals were similar to, or better than, those of abecarnil, and clearly superior to those of diazepam and clonazepam which

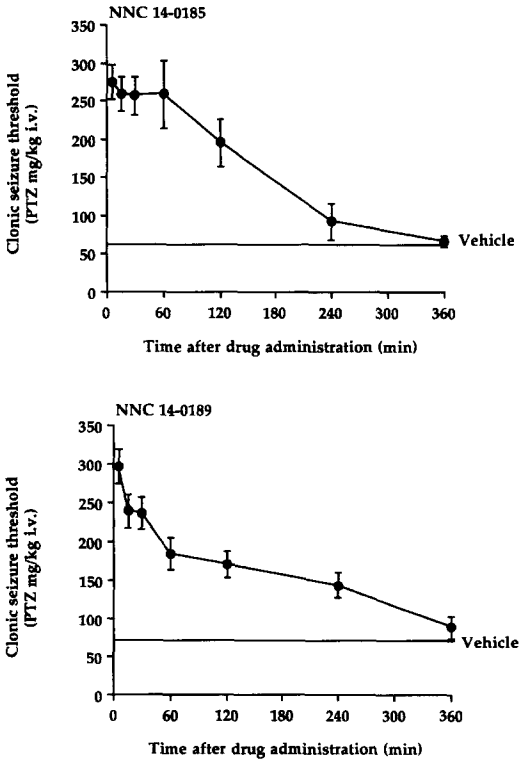

Fig. 3. Time course of the anticonvulsant effects of NNC 14-0185 and NNC 14-0189 in mice. Groups of 6–8 animals were injected i.p. with 10 mg/kg of the test drug and pentylenetetrazole (PTZ) seizure thresholds (means ± S.E.M.) were determined over the subsequent 6-h period. Seizure thresholds of vehicle-treated control animals were measured 5 and 360 min following injection.

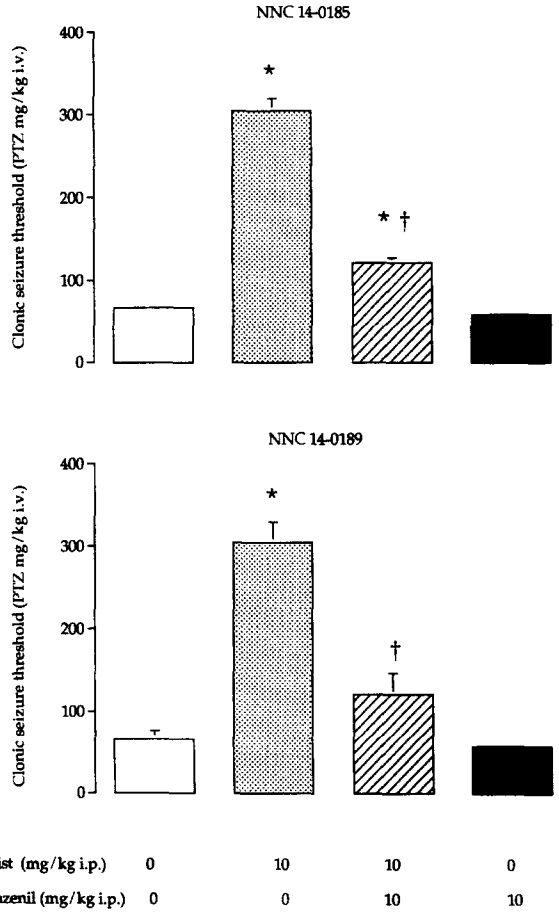

Fig. 4. Effect of flumazenil on the anticonvulsant activity of NNC 14-0185 and NNC 14-0189 in the pentylenetetrazole (PTZ) infusion test in mice. The results are expressed as means and S.E.M. for groups of 8 mice. Significant differences from the vehicle-treated group are shown by * $P < 0.05$. Significant differences from the animals given NNC 14-0185 or NNC 14-0189 alone are denoted by † $P < 0.05$.

produced muscle relaxation at anticonvulsant doses (Fig. 5).

NNC 14-0185 and NNC 14-0189 dose dependently prevented clonic seizures produced by electrical stimulation of the amygdala in kindled rats (Fig. 6). The ED_{50} values of NNC 14-0185 and NNC 14-0189 against the secondary generalisation of seizures (stage 3 to 5 seizures on the Racine scale; Racine, 1972) in this model of epilepsy were 0.4 and 0.9 mg/kg i.p. respectively, i.e. the compounds were as potent as abecarnil (ED_{50} = 0.6 mg/kg i.p.) and slightly more potent than diazepam (ED_{50} = 1.7 mg/kg i.p.). The effects of NNC 14-0185, NNC 14-0189, diazepam and abecarnil on rotarod performance in amygdala-kindled rats are also shown in Fig. 6. The ED_{50} values were 1, 3.2, 2.1 and 0.5 mg/kg i.p. respectively. Thus, the therapeutic windows between the anticonvulsant and ataxic effects of NNC 14-0185 and NNC 14-0189 in kindled rats were greater than those of diazepam and abecarnil (Table 2).

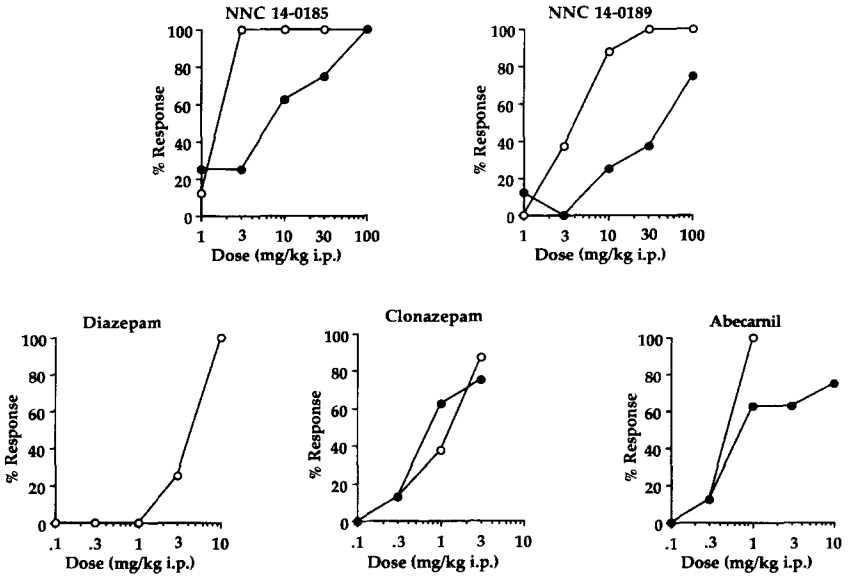

Fig. 5. The anticonvulsant and ataxic effects of NNC 14-0185, NNC 14-0189 and reference benzodiazepine receptor agonists in rats. The results are expressed as % response i.e., the % of animals protected against the clonic seizures induced by pentylenetetrazole (open circles) and the % of animals showing rotarod impairment (closed circles). Groups of 8 rats were injected with vehicle or the test drug 30 min prior to pentylenetetrazole (170 mg/kg s.c.). Rotarod performance was assessed a few minutes before seizure testing. Pentylenetetrazole produced clonic convulsions in 100% of the vehicle-treated animals and all the animals in this group were able to maintain their balance on the rotarod for the 2-min test period. The anticonvulsant and ataxic dose-response curves for diazepam were identical.

Tolerance did not develop to the anticonvulsant effects of NNC 14-0185 (10 mg/kg i.p.) and NNC 14-0189 (10 mg/kg i.p.) against pentylenetetrazole-induced seizures in mice as shown in Table 3. These compounds produced similar large increases in seizure threshold in mice treated repeatedly for three days with vehicle or test drug. Similar findings were observed for clonazepam (0.3 mg/kg i.p.) i.e., tolerance did not occur to its anticonvulsant effects in the 4-day test. In contrast, tolerance developed to the anticonvulsant effects of diazepam (10 mg/kg i.p.) and abecarnil (10 mg/kg i.p.) in this model. The increases in pentylenetetrazole seizure thresholds of mice treated chronically with diazepam or abecarnil were significantly lower (by about 30–40%) than those of the corresponding acute groups.

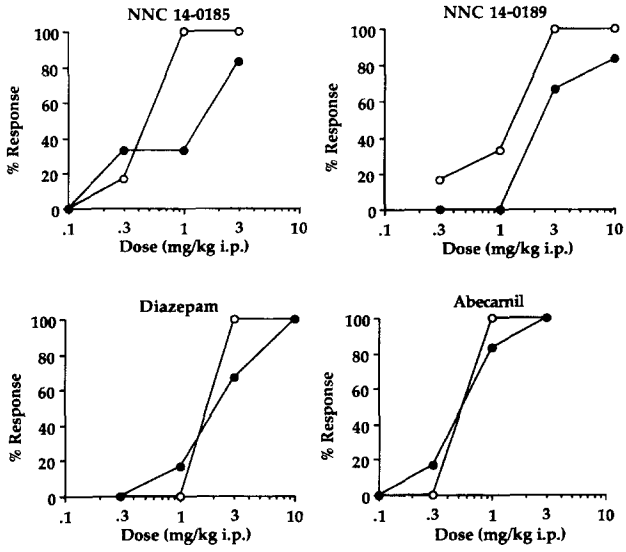

Fig. 6. The anticonvulsant and ataxic effects of NNC 14-0185, NNC 14-0189, diazepam and abecarnil in amygdala-kindled rats. The results are expressed as the percentage response i.e., the percentage of animals protected against the secondary generalization of seizures (stage 3–5 in the Racine scale) induced by electrical stimulation of the amygdala (open circles) and the % of animals showing rotarod impairment (closed circles). Treatment groups contained 5–7 rats. Animals were injected with the test drug 30 min before exposure to the electrical current. Rotarod performance was assessed a few minutes before seizure testing.

4. Discussion

The major finding of this study was that NNC 14-0185 and NNC 14-0189 are potent and efficacious anticonvulsants in mice and rats, with an improved overall side-effect profile when compared with the benzodiazepine receptor agonists currently used to treat epilepsy.

NNC 14-0185 and NNC 14-0189 protected against audiogenic-induced seizures in DBA/2 mice with potencies similar to those of diazepam and clonazepam. This sensitive model of reflex epilepsy gives little information about the mechanism of action of anticonvulsant drugs. However, NNC 14-0185 and NNC 14-0189 would appear to be benzodiazepine receptor agonists since they have high affinity for benzodiazepine receptors (see Introduction) and prevented seizures induced by convulsants interfering with GABAergic function, including pentylenetetra-

Table 3
Effects of NNC 14-0185, NNC 14-0189 and reference benzodiazepine receptor agonists in a four-day tolerance test in mice

Treatment	Dose	Acute seizure threshold	Chronic seizure threshold	% acute effect
NNC 14-0185	Baseline	65 ± 8	–	
	10 mg/kg i.p.	200 ± 29	219 ± 17	114
NNC 14-0189	Baseline	76 ± 7	–	
	10 mg/kg i.p.	228 ± 22	224 ± 22	98
Diazepam	Baseline	56 ± 4	–	
	10 mg/kg i.p.	219 ± 14	159 ± 8 [a]	63
Clonazepam	Baseline	62 ± 6	–	
	0.3 mg/kg i.p.	195 ± 11	177 ± 17	87
Abecarnil	Baseline	60 ± 3	–	
	3 mg/kg i.p.	211 ± 18	162 ± 11 [a]	68

Acute and chronic seizure thresholds (mg/kg pentylenetetrazole i.v.) are expressed as means ± S.E.M. for groups of 10 mice. Baseline values are the clonic seizure thresholds of animals treated with vehicle throughout the experiment. The acute group received vehicle twice a day for three days and the drug on day 4. The chronic group received drug twice a day for three days and again on day 4. Chronic seizure thresholds are also expressed as % of the increase in seizure threshold following acute drug administration (baseline seizure thresholds were subtracted from both the acute and chronic seizure thresholds before the calculation was performed). [a] $P < 0.05$ compared to acute seizure threshold.

zole, bicuculline, DMCM and 3-mercaptopropionic acid-induced seizures in mice and pentylenetetrazole-induced seizures in rats. Moreover, the increase in pentylenetetrazole seizure threshold produced by NNC 14-0185 and NNC 14-0189 in mice was inhibited by the benzodiazepine receptor antagonist, flumazenil.

The anticonvulsant effects of NNC 14-0185 and NNC 14-0189 against pentylenetetrazole-induced seizures in mice were apparent within 5 min and were still evident 2–4 h after injection i.e., the anticonvulsant duration of action of these compounds was similar to that of diazepam and abecarnil.

In general, NNC 14-0185 and NNC 14-0189 were equipotent with the reference benzodiazepine receptor agonists against seizures induced by the chemical convulsants. However, some interesting differences were observed. For instance, NNC 14-0185 and NNC 14-0189, were most potent against the seizures produced by DMCM in mice, whereas diazepam and clonazepam were most potent against the seizures induced by the $GABA_A$ receptor antagonist, bicuculline. Indeed, NNC 14-0185 and NNC 14-0189 were inactive against the seizures induced by a high dose of bicuculline and, unlike diazepam, failed to prevent the tonic hindlimb extension produced by maximal electroshock in mice. Abecarnil also potently prevented DMCM-induced seizures but was inactive against the seizures induced by a high dose of bicuculline and maximal electroshock (this study and Turski et al., 1990). Thus, the anticonvulsant profiles of NNC 14-0185, NNC 14-0189 and abecarnil were very similar. The different potencies of these compounds against the seizures induced by different chemical convulsants and electroshock could be due to partial agonism and/or subtype selectivity (see below).

The potential antiepileptic effects of NNC 14-0185 and NNC 14-0189 were confirmed in amygdala-kindled rats – a model of complex partial epilepsy with secondary generalization (Löscher et al., 1986). The imidazoquinazolines were equipotent with abecarnil for preventing the clonic convulsions induced by electrical stimulation of the amygdala and all three compounds were more potent than diazepam in this respect.

NNC 14-0185, NNC 14-0189 and abecarnil had much better therapeutic windows in both rats and mice than either diazepam or clonazepam. Our results with abecarnil are consistent with reports by others showing that it is anticonvulsant (and anxiolytic) in experimental animals at doses that do not produce muscle relaxation or ataxia (Löscher et al., 1990; Stephens et al., 1990; Turski et al., 1990; Ozawa et al., 1994a). The therapeutic index of NNC 14-0185 was comparable to that of abecarnil, whereas the separation between the anticonvulsant and muscle relaxant effects of NNC 14-0189 was, in general, twice that of abecarnil.

Two concepts may help to explain the improved therapeutic potential of compounds such as NNC 14-0185, NNC 14-0189 and abecarnil. The first is that of partial agonism. There is some evidence that the anticonvulsant actions of benzodiazepines emerge at lower levels of receptor occupancy than do their sedative and muscle relaxant effects (Jensen and Petersen, 1983). The pharmacological effects of partial agonists require higher levels of receptor occupancy than do those of full agonists. It follows therefore that partial agonists may be anticonvulsant without producing sedation and ataxia, even when all the receptors are fully occupied (Haefely and Polc, 1986; Haefely et al., 1992). Furthermore, low-efficacy agonists may be less likely to produce tolerance and dependence than full agonists (Miller et al., 1990; Moreau et al., 1990). The second concept is based on $GABA_A$ receptor hetereogeneity (see Mohler et al., 1995). Molecular pharmacology has shown that the $GABA_A$ receptor complex is comprised of 5 protein subunits. At least 16 different subunits exist (α_{1-6}, β_{1-4}, γ_{1-3}, ρ_{1-2}, δ_1) which can combine in a variety of different ways. The mRNAs of the different $GABA_A$ receptor subunits exhibit distinct distribution patterns in the central nervous system (Seeburg et al., 1990;

Persohn et al., 1992; Wisden et al., 1993). Therefore it may be possible to develop drugs that target particular $GABA_A$ receptors in particular parts of the brain. Such drugs may not have the adverse effects arising from activation of receptors in other brain regions or the spinal cord.

Abecarnil acts as a partial agonist in some biochemical and behavioural tests but as a full agonist in others (Turski et al., 1990; Stephens et al., 1990, 1992). One possible explanation for this is that it may possess varying degrees of intrinsic activity at the different subtypes of $GABA_A$ receptor. Electrophysiological studies provide some evidence in support of this hypothesis. For instance, abecarnil has been shown to act as a full agonist at recombinant receptors containing the α_1- and α_3-subunit and as a partial agonist at receptors containing the α_2- or α_5-subunit (Pribilla et al., 1993; Knoflach et al., 1993).

The subtype selectivity of NNC 14-0185 and NNC 14-0189 has not been fully evaluated. However, the similarities between the distinct anticonvulsant and side-effect profiles of NNC 14-0185, NNC 14-0189 and abecarnil suggest that it would be pertinent to compare the affinities and intrinsic activities of these compounds at receptors containing α_1-, α_2-, α_3- and α_5-subunits. Such studies would help to test the hypothesis that the side-effects of benzodiazepines are mediated by receptors containing α_2- and α_5-subunits (Mohler et al., 1995). In addition, they may help to explain the differential effects of the agonists against the seizures induced by the different chemical convulsants which may also act, either directly or indirectly, at $GABA_A$ receptor subtypes (e.g. see White and Gurley, 1995).

Partial agonist properties of NNC 14-0185 and NNC 14-0189 have been observed in some tests. For example, NNC 14-0185 and NNC 14-0189 enhance binding of the $GABA_A$ receptor antagonist cage convulsant, [^{35}S]*t*-butylbicyclophosphorothionate ([^{35}S]TBPS), by 132 and 147%, respectively compared with 159% for diazepam (unpublished observations; P. Suzdak). In addition, whereas diazepam and other full agonists produce marked decreases in body temperature in mice, NNC 14-0185 had no effect on body temperature, and NNC 14-0189 produced a relatively small hypothermic response i.e., they acted in a similar manner to other partial agonists (unpublished data; Jackson and Nutt, 1990).

Tolerance did not develop to the increase in pentylenetetrazole seizure threshold induced by NNC 14-0185 and NNC 14-0189 in mice as shown in the current study. Similar results were obtained with clonazepam. However, an important difference between clonazepam and the imidazoquinazolines was observed. The dose of clonazepam used in the tolerance test (0.3 mg/kg) produced rotarod impairment in 50% of the animals tested whereas NNC 14-0185 and NNC 14-0189 did not produce muscle relaxation until doses 3–5-fold greater than those used in the tolerance studies. The pentylenetetrazole tolerance test used in this study was modified from that originally described by Gent et al. (1984). These authors observed that some tolerance occurred to the anticonvulsant action of clonazepam, however, in agreement with our results, the development of tolerance to clonazepam was reduced in comparison with that induced by benzodiazepine agonists such as diazepam and clobazam (Gent et al., 1985; Haigh and Feely, 1988).

In the current study, tolerance developed to the anticonvulsant effects of the full agonist, diazepam, and the novel β-carboline, abecarnil. Tolerance to the anticonvulsant effects of abecarnil under some conditions has been reported by other workers (Schneider et al., 1990; Steppuhn, 1992 and see Löscher et al., 1991) although, in general, it has been found to have a low propensity to produce tolerance and dependence (Löscher et al., 1990; Steppuhn et al., 1993, Ozawa et al., 1994b; Serra et al., 1994). Further studies are required to confirm our interesting findings that NNC 14-0185 and NNC 14-0189 have less tolerance liability than diazepam, and particularly abecarnil, and to evaluate the effects of these compounds in animal models of dependence. Pharmacokinetic factors should also be determined and taken into consideration as they may influence results obtained following chronic drug administration.

To summarize, we have shown that NNC 14-0185 and NNC 14-0189 are efficacious anticonvulsants in amygdala-kindled rats and against the clonic seizures induced by sound and a variety of chemical convulsants in mice and rats. These effects appear to be mediated by benzodiazepine receptors. NNC 14-0185 and NNC 14-0189 displayed much better separation between their anticonvulsant and ataxic effects than either diazepam and clonazepam and in some cases possessed better therapeutic windows than the novel β-carboline, abecarnil. Tolerance did not develop to the anticonvulsant action of NNC 14-0185 and NNC 14-0189 over a 4-day period. In conclusion, NNC 14-0185 and NNC 14-0189 appear to have advantages over the benzodiazepines currently used to treat epilepsy and their potential as novel anticonvulsant agents should be explored further.

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