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European Journal of Pharmacology 285 (1995) 289–297

ejp

Differentiation of in vivo effects of AMPA and NMDA receptor ligands using drug discrimination methods and convulsant/anticonvulsant activity

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Received 6 February 1995; revised 1 June 1995; accepted 11 July 1995

Abstract

The discriminative stimulus properties of the AMPA ((*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid) receptor agonist ATPA ((*RS*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propionic acid) and NMDA (*N*-methyl-D-aspartic acid) in rats have been characterized. It is suggested that the cues are mediated by separate mechanisms in the central nervous system. The ATPA cue is not mimicked by NMDA or an NMDA receptor agonist, and is inhibited by the AMPA receptor antagonist (*R*)-APPA ((*R*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid) but not the AMPA receptor antagonist ATOA ((*RS*)-2-amino-3-(3-carboxymethoxy-5-*tert*-butylisoxazol-4-yl)propionic acid) or the NMDA receptor antagonist CPP ((*RS*)-3-(2-carboxypiperazin-4-yl)propyl)phosphonic acid). The ATPA cue is not mimicked by AMPA which is believed not to penetrate the blood-brain barrier. In contrast, ATPA does not generalize to the NMDA cue, which is mimicked by some NMDA receptor agonists (tetrazol-5-yl-glycine and AMAA ((*RS*)-2-amino-2-(3-hydroxy-5-methylisoxazol-4-yl)acetic acid)) and is inhibited by the NMDA receptor antagonist CPP. Highly potent convulsant activity was demonstrated in mice with all AMPA and NMDA receptor agonists after intracerebroventricular (i.c.v.) injection, whereas weaker or no effects were found after subcutaneous (s.c.) or intravenous injection. Only (*RS*)-tetrazol-5-yl-glycine had a potent effect after s.c. administration. I.c.v. ATOA and CPP inhibited convulsions induced by i.c.v. injection of AMPA or NMDA, while (*R*)-APPA was ineffective. These results indicate that there are differences in the structure-activity relations in the drug discrimination and convulsant/anticonvulsant models, even when effects after i.c.v. and s.c. injection are taken into consideration. The convulsion models are rapid tests which can give an indication of central nervous system penetration, but are less pharmacologically specific with respect to differentiation between AMPA and NMDA ligands where cue models demonstrate clear differences in effects of ligands with selectivity for receptor subtypes.

Keywords: AMPA ((*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid); NMDA (*N*-methyl-D-aspartate); ATPA ((*RS*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propionic acid); Cue; Convulsion; Glutamate receptor; (Rat); (Mouse)

1. Introduction

Glutamate receptors are divided into a variety of subtypes among which the NMDA (*N*-methyl-D-aspartate) and AMPA ((*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid) subtypes are among the most thoroughly characterized (Brehm et al., 1988; Monaghan et al., 1989; Watkins et al., 1990; Cunningham et al., 1994, for reviews).

Although a wealth of information is available on the structure-activity relationship of agonists and antagonists in binding studies and functional in vitro systems, very few animal models are available for characterization of NMDA and AMPA receptor ligands in vivo.

Drug discrimination procedures have proved to be very useful for studying mechanisms of drug action in

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SSDI 0014-2999(95)00422-X

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1. Introduction

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Although a wealth of information is available on the structure-activity relationship of agonists and antagonists in binding studies and functional in vitro systems, very few animal models are available for characterization of NMDA and AMPA receptor ligands in vivo.

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SSDI 0014-2999(95)00422-X

vivo and have been used extensively to characterize a variety of neurotransmitter receptors and subtypes in the central nervous system (CNS) (Colpaert and Balster, 1988, for review). Relatively few studies have investigated the discriminative stimulus properties of ligands. NMDA discrimination has been clearly demonstrated and thoroughly characterized (Amrick and Bennett, 1987; Koek et al., 1990; Willetts and Balster, 1989; Grech et al., 1993) but the effects of AMPA receptor ligands in NMDA-trained rats have not yet been reported.

Most drug discrimination studies of NMDA receptor ligands have focussed on the stimulus effects of the non-competitive antagonists PCP (phencyclidine) and MK-801 (5-methyl-10,11-dihydro-5*H*-dibenzo-[*a*,*d*]cyclohepten-5,10-imine; Tricklebank et al., 1989; Koek et al., 1990). No studies have examined the discriminative stimulus properties of AMPA receptor ligands, probably due to lack of suitable training compounds. In the present study the stimulus effects of the AMPA receptor agonist ATPA ((*RS*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propionic acid; Lauridsen et al., 1985; Ebert et al., 1992) are described and compared with the stimulus effects of NMDA.

Furthermore, NMDA and AMPA receptor ligands are examined for their convulsant/anticonvulsant effects after systemic and intraventricular administration in mice, an attempt to evaluate the CNS penetration of the test compounds (Lehmann et al., 1988; Chapman and Meldrum, 1989; Koek and Colpaert, 1990; Bisaga et al., 1993). Preliminary characterization of ATPA discrimination has been reported in abstract form earlier (Arnt et al., 1992; Swedberg and Jacobsen, 1992). Preliminary studies of convulsant/anticonvulsant activity have been presented at the Epilepsia Meeting, Vancouver 1993 by Connie Sánchez.

2. Materials and methods

2.1. Animals

Male Wistar rats (Mol:Wist strain, Møllegaard, Denmark) weighing 150–200 g at the beginning of training were used for drug discrimination experiments. They were housed in groups of four in Macrolon type III cages in animal rooms at 21 ± 2°C with a relative humidity of 55 ± 10%, air exchange (16 times/h) and day/night cycle (light on 6 a.m.–6 p.m.). They had free access to commercial food pellets throughout the study, but water was available only at limited periods (see later).

Male mice (NMRI/BOM, SPF, Bomholtgård, Denmark) weighing 24–26 g were used for the studies of pro- or anticonvulsant effects. The mice were kept in groups of 20 in plastic cages (35 × 30 × 12 cm).

2.2. Drug discrimination procedures

Apparatus

Eight two-lever wire mesh boxes (29 × 24 × 19 cm) equipped with a dipper, located equidistant between the two levers, were used. The boxes were placed in sound-proofed chambers with ventilation fans providing a constant level of noise.

Discrimination learning

A procedure similar to that described by Nielsen and Jepsen (1985) was followed. Rats were deprived of water by restricting water intake to that received as reward in the training box. In the initial training period only one of the levers was accessible and the rats were shaped to respond under gradually increasing fixed ratio (FR) schedules for water reward (0.1 ml) up to FR32. The FR schedules were run as FR1, FR3, FR6, FR12, FR20, and FR32 for saline *and* drug training sessions. Hereafter, rats had access to both levers during all sessions. Session times were 45 min until FR6, when they were reduced to 30 min, because of gradually increasing response rates. The criterion was that each rat obtained 50–100 rewards in one training session, i.e. 5–10 ml of water. Fifteen minutes before the start of the session the rats received an i.p. injection of either drug or saline. The trial began 20 s after the rat was placed in the box, when the house-light was switched on. After injection of saline, only responses on a designated lever (saline lever) were rewarded and after drug only responses on the opposite lever (drug lever) were rewarded. Incorrect responses had no consequences. Drug and saline levers were randomly allocated to the left and right for different rats. When stable rates of responding were obtained after about 2 weeks of discrimination training with the FR32 schedule (and both levers accessible), the session time was reduced to 20 min. Again, the criterion was that each rat obtained 50–100 rewards during the session. Free access to water was given for 1 day after the training session on Friday or Saturday. The order of saline and drug training sessions was as follows. In the beginning of discrimination training, saline or drug trials were given for 2 successive days each. When discrimination accuracy gradually increased, saline and drug sessions were interchanged daily, although this regular pattern was now and then broken by 2 identical training days in a row. The dose of each training drug was as follows: NMDA 40 mg/kg = 270 μmol/kg i.p. and ATPA 5 mg/kg = 22 μmol/kg i.p. The level of discrimination accuracy was expressed as the percentage correct responses before the first reward on the lever appropriate after injection of drug or saline. Latency time was defined as the time until the first reward was obtained.

Drug testing

When the rats in at least 8 out of 10 concecutive training sessions (5–6 days a weak) showed accuracy of discrimination, corresponding to the criterion given below, test trials were started. In the NMDA group some rats developed small clonic convulsions after 11 months training. Therefore, the dose of NMDA was reduced to 30 mg/kg which did not reduce stimulus control. However, convulsions were no longer seen. A dose-response of NMDA before and after adjustment of training dose indicated no significant change in sensitivity of the discriminative stimulus. The minimum criterion for accuracy was at least 90% correct discrimination for the group as a mean and at least 75% correct responding for individual animals in saline and drug training sessions. However, the observed accuracy was generally at least 95% correct responding. If an insufficient number of rats attained the criterion, extra training trials were given before the test session. A rat that did not reach the required accuracy in saline and drug training sessions was omitted from the test and received further training. Training trials with drug and saline were always included between test sessions. Thus, one to two tests were done weekly. In tests for antagonistic effects, drugs were injected subcutaneously (s.c.) at the indicated time before administration of the usual training dose of drug (for pretreatment times, see figure and table legends). In tests for agonist effects, the test compound was substituted for the training drug. The number of rats in each group is indicated in the figure legends. In each experiment five to eight rats were used. Some test doses were given again to ensure reproducibility and the results were pooled.

The test trial was terminated when the number of responses (32) corresponding to the FR schedule were made on either lever or when 20 min had elapsed. No rewards were given, and the rats were removed from the test box immediately and received no further training on the test day. Free access to water was provided for 15 min in the home cage, about 1 h after completion of the test trial. Only data from rats making at least 10 responses on one lever were included in the data analysis. Furthermore, only dose groups in which at least half of the rats responded (and at least four rats) are shown in dose-response curves. The time to complete the test trial was recorded as latency time. If less than 32 responses were made on one lever, the latency time was assigned the total length of the trial (1200 s).

Calculations

ED_{50} values with 95% confidence limits were calculated by log-probit analyses as the dose producing 50% drug-relevant response (test for agonistic effect) or 50% inhibition of drug-lever responding (test for antagonistic effect). Confidence limits (95%) of ED_{50} values are presented as deviation factors and are defined as the ratio between the upper confidence limit and the ED_{50} value. For compounds with a biphasic effect, the ascending part of the dose-response curve was used for determination of the ED_{50} value. Response data, calculated as percentage of drug lever responses, were compared with Wilcoxon's matched pairs signed rank test, using the preceding training trial (drug or saline) as control. Because of the near-maximal accuracy in control trials, a one-tailed distribution was relevant for calculation of significance levels. Latency time data were analyzed by means of a *t*-test. Two control sessions before and after the test trial were used to obtain a symmetric comparison. This was done separately for saline and drug training trials. Raw data were logarithmically transformed to obtain homogeneity of variances and a normal distribution of latency times. The mean (X) and standard deviation (S_x) of the four logarithmically transformed control values were then calculated. The transformed latency time in the test trial (Y) was subsequently compared with that of the four control trials using a *t*-test with three degrees of freedom:

$$t = (Y - X)/S_x\sqrt{1 + 1/n}$$

where n (= 4) is the number of control sessions used in the calculation. A two-tailed distribution was used to calculate significance levels.

2.3. Convulsant / anticonvulsant activity

Each dose group consisted of 5 mice and a total of 2–3 experiments with overlapping doses were performed for each drug. ED_{50} values were calculated by log-probit analysis.

Chemically induced convulsions

Drugs were given intravenously (i.v., 10 ml/kg), subcutaneously (s.c., 10 ml/kg) or intracerebroventricular (i.c.v., 5 μl/30 s/mouse) into the third ventricle and the animals were observed for up to 1 h for presence or absence of clonic/tonic convulsions. The i.c.v. administration was performed by means of a 0.5 mm needle connected by a polyethylene tubing to a 100 μl Hamilton syringe placed in a micropump (Microject, Carnegie Medicine, Sweden). A piece of plastic catheter allowed for only 3.4 mm of the needle to pass the skull. The skull perforation was done free hand by pressing the mouse upward so that the needle perforated the skull in the midline 1–2 mm caudal to the eyes as described by Haley and McCormick (1957), The mouse was removed a few seconds after the infusion was completed.

Antagonism of NMDA- or AMPA-induced convulsions. In these experiments NMDA (0.054 μmol/kg) or AMPA (0.30 μmol/kg) was coadministered with the

antagonist by i.c.v. injection (5 μl/mouse) into the third ventricle as described above.

2.4. Drugs

The following drugs were dissolved in minimal amounts of 0.1 N NaOH and, if necessary, neutralized with diluted hydrochloric acid to neutral pH:

ATPA hydrate ((*RS*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propionic acid; molecular weight (MW) 228); (*RS*)-tetrazol-5-yl-glycine (MW 143; Tocris); *cis*-2,4-methanoglutamate (MW 177; Tocris); AMAA ((*RS*)-2-amino-2-(3-hydroxy-5-methyl-isoxazol-4-yl)acetic acid; MW 190); ATOA ((*RS*)-2-amino-3-(3-carboxymethoxy-5-*tert*-butyl-isoxazol-4-yl)propionic acid; MW 286); NMDA (*N*-methyl-D-aspartic acid; MW 147; Sigma) and CNQX (6-cyano-7-nitroquinoxaline-dione; MW 232; Tocris).

The following drugs were dissolved in saline: AMPA hydrobromide ((*RS*)-2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propionic acid; MW 267; Research Biochemicals) and CPP hydrate ((*RS*)-3-(2-carboxypiperazin-4-yl)propyl)phosphonic acid; MW 288; Tocris). (*S*)-APPA ((*S*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid; MW 248; H. Lundbeck). (*R*)-APPA ((*R*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid; MW 266; H. Lundbeck) were dissolved by addition of deionized water (1/3 of final volume), stirring slowly for 5–10 min, and slowly adding 0.1 M sodium bicarbonate until pH reached 6.95–7.0. Again the mixture was stirred for about 5 min and finally diluted to volume with deionized water. Compounds for which a commercial source are not mentioned have been synthesized at the Department of Medicinal Chemistry, The Royal Danish School of Pharmacy, Copenhagen, Denmark.

Injection volumes were 5 μl/mouse (i.c.v.) or 10 ml/kg in mice and 1 ml/kg in rats. For test compounds given in 20 mg/kg or higher doses to rats it was necessary to give 2 ml/kg body weight due to solubility limitations.

3. Results

3.1. Drug discrimination

ATPA (5 mg/kg i.p.) discrimination was acquired more easily than that of NMDA (40 mg/kg i.p.) as illustrated in the time-course curves of discrimination accuracy (Fig. 1). Furthermore, response rates (measured as latency time to the first reward) were similar after ATPA and saline training trials, while NMDA prolonged latency times compared with saline (Fig. 1).

In ATPA-trained rats no convulsions appeared within more than one year of training and testing in contrast to the observation of mild clonic convulsions in some NMDA-trained rats (see Materials and methods).

The stimulus effect of NMDA and ATPA was short lasting: ATPA induced 61 and 17% drug responding, whereas NMDA induced 43 and 24% drug responding 30 and 60 min after injection, respectively (data not shown).

The dose-response generalization curves of ATPA and NMDA in both training groups are illustrated in Fig. 2. In ATPA-trained rats, dose-dependent generalization is observed with ATPA (10–41 μmol/kg = 2.5–10 mg/kg), while NMDA (2.1–140 μmol/kg = 0.31–20 mg/kg) produced no more than 40% substitution. Latencies are prolonged at the 2 higher doses of NMDA (68 and 140 μmol/kg s.c.). In NMDA trained rats the reverse result is obtained. NMDA (68–270 μmol/kg = 10–40 mg/kg) induces dose-dependent generalization,

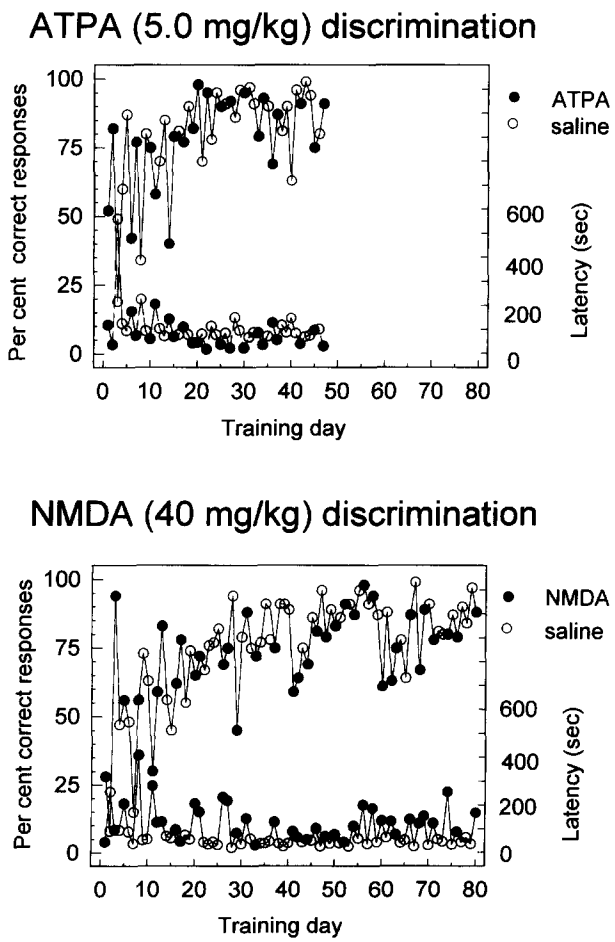

Fig. 1. Acquisition of the discriminative stimulus properties of ATPA (5.0 mg/kg i.p.) and NMDA (40 mg/kg i.p.) in rats. Day 1 indicates the first training session in which both levers are presented. Training was conducted 5–6 days a week. On the left ordinate the percentage correct lever responses are shown (upper graphs) for drug (•) or saline (○) trials. On the right ordinate the latency time (s) to complete the first fixed ratio schedule, i.e. 32 correct responses, is shown (lower graphs). Eight rats were included in each group.

whereas ATPA (20–81 μmol/kg = 5–20 mg/kg) has marked disruptive effects on lever responding. At an ATPA dose of 81 μmol/kg 3 out of 6 rats did not respond within 20 min. Relative potencies of ATPA and NMDA in the 2 training groups are indicated as ED_{50} values in Table 1.

Potencies of a variety of glutamate agonists and antagonists are shown in Figs. 2 and 3 and in Table 1, and the dose-response of the NMDA receptor antagonist CPP ((*RS*)-3-(2-carboxypiperazin-4-yl)propyl)phosphonic acid) and the AMPA receptor antagonist (*R*)-APPA ((*R*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid) are shown in Fig. 4.

AMPA (19–37 μmol/kg = 5–10 mg/kg) and the AMPA receptor agonist (*S*)-APPA ((*S*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid; 40–320 μmol/kg = 10–80 mg/kg); Ebert et al., 1994) fail to generalize to the ATPA stimulus. The only effect of AMPA is to produce some response disruption (Fig. 3).

The NMDA receptor agonist (*RS*)-tetrazol-5-yl-glycine (0.28–2.2 μmol/kg = 0.04–0.31 mg/kg; Schoepp et al., 1991) dose-dependently generalizes to the NMDA stimulus, while inducing response disruption in ATPA-trained rats (2.2 μmol/kg = 0.31 mg/kg). Further, the NMDA receptor agonist AMAA ((*RS*)-2-amino-2-(3-hydroxy-5-methyl-isoxazol-4-yl)-acetic acid; Madsen et al., 1990; 1.0–53 μmol/kg = 0.31–10 mg/kg) generalizes to the NMDA discrimination. In contrast, another NMDA receptor agonist, *cis*-2,4-methanoglutamate (Lanthorn et al., 1990; 31–63 μmol/kg = 5–10 mg/kg), does not substitute for

Table 1
Effects of AMPA and NMDA receptor agonists (substitution tests) and antagonists (inhibition tests) on the discriminative stimulus properties of ATPA (5 mg/kg) and NMDA (30 or 40 mg/kg)

Compound	ATPA-trained rats ED_{50} (μmol/kg s.c.)	NMDA-trained rats ED_{50} (μmol/kg s.c.)
AMPA agonist		
ATPA	15	> 41
AMPA	> 37	NT
(*S*)-APPA	> 320	NT
NMDA agonists		
NMDA	> 140	120
(*RS*)-Tetrazol-5-yl-glycine	> 1.1	0.98
cis-2,4-Methanoglutamate	NT	> 63
AMAA	NT	28
AMPA antagonists		
CNQX	> 43	NT
(*R*)-APPA	87	NT
ATOA	> 150	> 70
NMDA antagonists		
CPP	> 74	3.2

Results are summarized as ED_{50} values. For further details, see Materials and methods. All compounds were administered 30 min before start of test, except NMDA (15 min). NT: not tested.

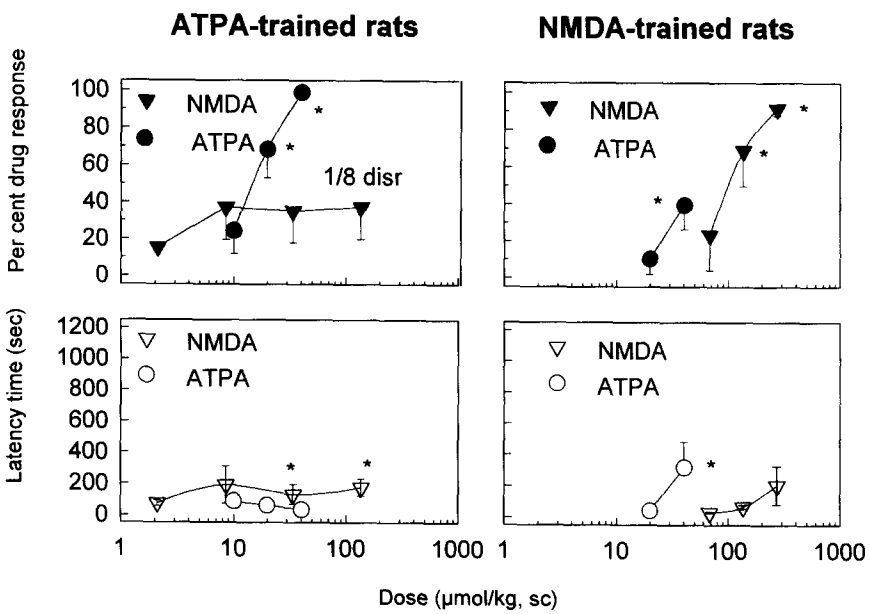

Fig. 2. Effect of ATPA (given 30 min before test) and NMDA (15 min before test) in rats trained to discriminate between saline and either ATPA (5.0 mg/kg) or NMDA (40 mg/kg). The results obtained in ATPA-trained rats are shown in the left panels and the results in NMDA-trained rats in the right panels. The upper graphs show the percent (mean ± S.E.M.; $n = 4–8$) responses on the lever appropriate for training drug. The lower graphs indicate latency times (mean ± S.E.M.; time to complete the fixed ratio 32 schedule on either lever) for the same rats. Fractional values on the upper graphs indicate the number of non-responding rats out of the total number of rats tested at the given dose. * Significant increase ($P < 0.05$) in percent drug responding (upper graphs) or increase in latency time (lower graphs) compared with saline control trials in the same rats (see Materials and methods).

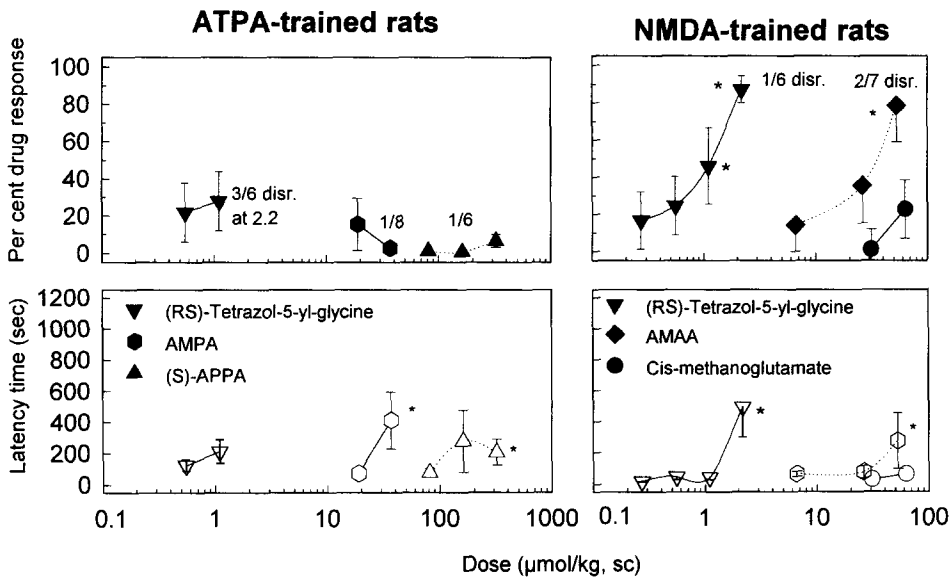

Fig. 3. Effect of (*RS*)-tetrazol-5-yl-glycine, AMPA, (*S*)-APPA, AMAA and *cis*-2,4-methanoglutamate (all given 30 min before) in rats trained to discriminate between saline and either ATPA (5.0 mg/kg) or NMDA (40 mg/kg). Only (*RS*)-tetrazol-5-yl-glycine was studied in both groups of rats. The results obtained in ATPA-trained rats are shown in the left panels and the results in NMDA-trained in the right panels. The upper graphs show the percent (mean ± S.E.M.; $n = 4–8$) responses on the lever appropriate for training drug. The lower graphs indicate latency times (mean ± S.E.M.; time to complete the fixed ratio 32 schedule on either lever) for the same rats. Fractional values in the upper graphs indicate the number of non-responding rats out of the total number of rats tested at the given dose. *Significant increase ($P < 0.05$) in percent drug responding (upper graphs) or increase in latency time (lower graphs) compared with saline control trials in the same rats (see Materials and methods).

NMDA (Fig. 3; Table 1). The two latter compounds were not tested in ATPA-trained rats.

Three putative AMPA receptor antagonists were studied in ATPA-trained rats. CNQX ((6-cyano-7-nitroquinoxalinedione; 11–43 μmol/kg = 2.5–10 mg/kg; Honoré et al., 1988) and ATOA (37–150 μmol/kg = 10–40 mg/kg; basic characterization to be published elsewhere) fail to antagonize the ATPA

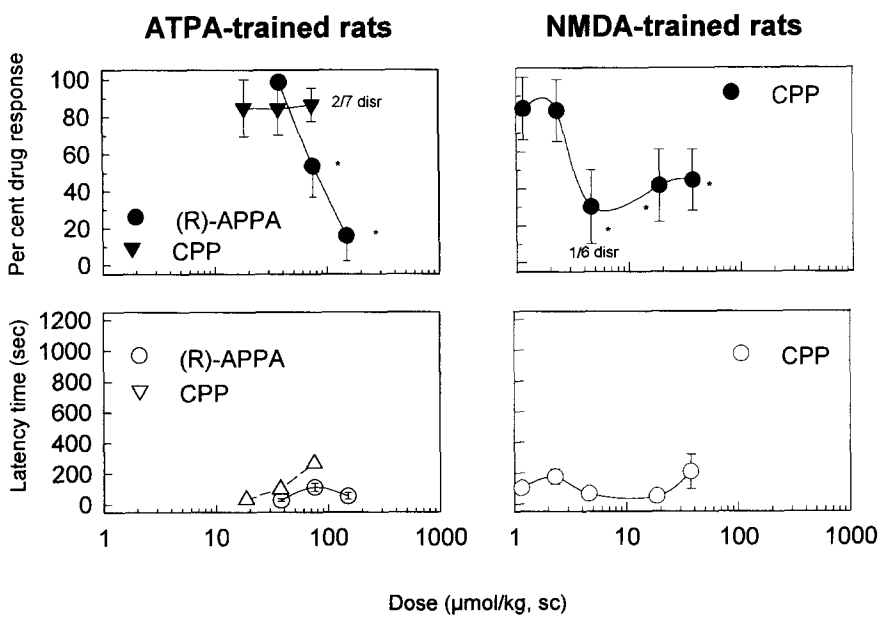

Fig. 4. Antagonistic effect of CPP and (*R*)-APPA in ATPA-trained rats (left) and of CPP in NMDA-trained rats (right). Antagonists were injected 15 min before the usual training dose of ATPA (5.0 mg/kg) and NMDA (40 mg/kg). For further explanation, see legend to Fig. 2. *Significant inhibition ($P < 0.05$) in percent drug responding (upper graphs) or increase in latency time (lower graphs) compared with drug control trials in the same rats (see Materials and methods).

stimulus (data not shown), whereas (*R*)-APPA ((*R*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid; Ebert et al., 1994; 38–150 μmol/kg = 10–40 mg/kg) induce dose-dependent antagonism at high doses (Fig. 4; Table 1).

The competitive NMDA receptor antagonist CPP dose-dependently (1.2–4.6 μmol/kg = 0.31–1.25 mg/kg) inhibits NMDA discrimination, but does not inhibit the effect of ATPA up to dosages inducing response disruption (19–74 μmol/kg = 5–20 mg/kg; Fig. 4; Table 1). No data for (*R*)-APPA in NMDA-trained rats are available.

3.2. Convulsant effects of AMPA and NMDA receptor agonists in mice

The results are summarized in Table 2.

After intravenous or subcutaneous administration high doses of AMPA and ATPA induce convulsions, whereas (*S*)-APPA is ineffective. NMDA (maximum dose 530 μmol/kg = 80 mg/kg) and *cis*-2,4-methanoglutamate (maximum dose 500 μmol/kg = 80 mg/kg) fail to induce convulsion after s.c. treatment, whereas (*RS*)-tetrazol-5-yl-glycine is a potent convulsant. These compounds are all effective when given intravenously.

After intracerebroventricular (i.c.v.) injection all studied glutamate receptor agonists are potent convulsants. Large potency ratios between i.c.v. and i.v. administration and even larger ratios between i.c.v. and s.c. administration are obtained, i.e. dose ratios between 9200 and 66000.

3.3. Inhibition of AMPA- and NMDA-induced convulsions in mice

The effects of i.c.v. injection of selected AMPA and NMDA receptor antagonists on convulsions induced by i.c.v. administration of AMPA or NMDA were studied in the subsequent experiments, which are summarized in Table 3.

The AMPA receptor antagonist ATOA blocks AMPA-induced convulsions and partially inhibits the effect of NMDA in the same dose range. In contrast, (*R*)-APPA is ineffective. The NMDA receptor antagonist CPP blocks NMDA-induced convulsions, while those induced by AMPA are only partially inhibited.

Table 2
Convulsive potencies of AMPA and NMDA receptor agonists after subcutaneous (s.c.), intravenous (i.v.) and intracerebroventricular (i.c.v.) administration in mice

	ED_{50} (μmol/kg)		
	s.c.	i.v.	i.c.v.
AMPA agonists			
AMPA	220	19	0.024
ATPA	260	190	0.029
(*S*)-APPA	> 320	> 320	0.39
NMDA agonists			
NMDA	> 530	96	0.0080
(*RS*)-Tetrazol-5-yl-glycine	5.3	2.7	0.0004
cis-2,4-Methanoglutamate	> 500	40	0.025

For details, see Materials and methods.

Table 3
Inhibition of AMPA-and NMDA-induced convulsions by intracerebroventricular (i.c.v.) administration of AMPA and NMDA receptor antagonists in mice

Compound	ED_{50} (μmol/kg i.c.v.)	
	AMPA	NMDA
AMPA antagonists		
(*R*)-APPA	> 6.5	> 6.5
ATOA	0.68	0.28 *
NMDA antagonists		
CPP	0.011 *	0.0020

AMPA (0.30 μmol/kg) or NMDA (0.054 μmol/kg) was administered i.c.v. with test compounds. * Maximum 60% protection.

4. Discussion

The results confirm that it is possible to obtain a reliable discrimination of NMDA or ATPA from saline and demonstrates that the AMPA receptor agonist, ATPA, also can be used as a discriminative stimulus. Furthermore, the results of generalization and antagonism experiments indicate that the discriminative stimuli are very different: NMDA and ATPA do not cross-generalize to each other, the NMDA receptor agonist (*RS*)-tetrazol-5-yl-glycine (Schoepp et al., 1991) generalizes to NMDA but induces response disruption in ATPA-trained rats and the NMDA receptor antagonist CPP selectively blocks NMDA discrimination, as described before (Amrick and Bennett, 1987; Koek et al., 1990; Willetts and Balster, 1989). In ATPA-trained rats CPP produced response disruption, but no inhibition of discrimination.

It proved difficult to characterize the ATPA discrimination in further detail, since most test compounds are ineffective. This includes the agonists AMPA and (*S*)-APPA (Ebert et al., 1994), as well as the antagonists CNQX (Sheardown et al., 1989) and ATOA while the weak antagonist (*R*)-APPA (Ebert et al., 1994) did block ATPA discrimination at high dosages. Furthermore, Swedberg and Jacobsen (1992) have failed to block ATPA discrimination with the AMPA receptor antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline; Honoré et al., 1988).

An important question is whether the stimulus effects of ATPA and NMDA can be regarded as centrally mediated. Although no direct evidence supports this hypothesis, it appears most likely for the following reasons: AMPA fails to generalize to ATPA when given in high and disruptive doses, although it is about 10 times more potent in the cortical slice preparation in vitro (Ebert et al., 1992) and is equipotent with ATPA to induce convulsion after intracerebroventricular injection in mice (Table 2). For NMDA-trained rats the indirect evidence for mediation by CNS sites comes from the lack of effect of *cis*-2,4-methanoglutamate, a potent NMDA receptor agonist in vitro but not in vivo (Lanthorn et al., 1990), whereas 2 other NMDA agonists, (*RS*)-tetrazol-5-yl-glycine (Schoepp et al., 1991) and AMAA (Madsen et al., 1990) are effective. The results are comparable with the activity profiles obtained in the tests for convulsive effects (see below). Furthermore, the NMDA receptor-antagonistic potency of CPP after systemic injection corresponds well to the potencies in vivo in anticonvulsant test models, while its potency is markedly higher after intracerebroventricular (i.c.v.) administration (present results; Koek and Colpaert, 1990; Patel et al., 1988).

The results indicate that all tested glutamate agonists are potent convulsants when administered directly into the brain ventricles, whereas much lower potencies are observed after intravenous or subcutaneous administration. This suggests that the compounds penetrate the blood-brain barrier poorly in mice. Whether similar profiles are relevant in rats is not known, but the weak drug potencies obtained in discrimination studies suggest relatively poor CNS penetration in this species, too. The large differences in convulsant potencies between intravenous and subcutaneous administration of some glutamate agonists (e.g. AMPA, NMDA and *cis*-2,4-methanoglutamate) also suggest a rapid biotransformation or excretion of these compounds. NMDA is known to induce convulsions in mice after intraperitoneal injection, but only in very high doses (100–250 mg/kg ~ 680–1700 μmol/kg; e.g. Bisaga et al., 1993). Thus the lack of effect of NMDA in the present study is mainly a consequence of administration of a subconvulsant dose (530 μmol/kg ~ 80 mg/kg). With the limitations in the comparison between results in mice and rats in mind it is noted that (*RS*)-tetrazol-5-yl-glycine is the most potent agonist in either species.

In order to investigate whether AMPA and NMDA receptor antagonists can be pharmacologically differentiated also in anticonvulsant test models the effects of selected AMPA and NMDA receptor antagonists on convulsions induced by i.c.v. administration of AMPA or NMDA in mice were studied. A partial differentiation can be achieved: the NMDA receptor antagonist CPP preferentially, but not specifically, blocks the NMDA-induced convulsions, while the AMPA receptor antagonist ATOA has the opposite activity profile. In contrast (*R*)-APPA was not found effective. These results indicate that NMDA and AMPA receptor mediated convulsions to some extent can be differentiated, although the pharmacological specificity is limited.

It is puzzling that some AMPA receptor agonists and antagonists behave differently in drug discrimination and convulsant/anticonvulsant models: (*S*)-APPA induces convulsions after i.c.v., but not after systemic injection, and fails to generalize to ATPA discrimination; ATOA fails to block ATPA discrimination, but inhibits AMPA-induced convulsions (after i.c.v. administration) and (*R*)-APPA inhibits ATPA discrimination, but fails to block AMPA-induced convulsions.

Whether these discrepancies depend on species differences between mice and rats (e.g. including differences in penetration of the blood-brain barrier) or is a result of their relatively weak potencies in vitro (Ebert et al., 1994) remains to be clarified. The observations also reflect differences in the mechanisms involved in the models used. Another possibility to consider is that AMPA (and NMDA) receptors are further divided into several subtypes with expected differences in pharmacology (Cunningham et al., 1994). Subtype selectivity of compounds used in this study for AMPA and NMDA receptors is presently unknown.

In summary, it can be concluded that drug discrimination can be used for in vivo evaluation of glutamate ligands, although compounds with better CNS penetration urgently are needed for further validation of the models. The convulsant/anticonvulsant profiling is also useful, but less pharmacological selectivity is obtained.

Acknowledgements

The authors thank Hanne Albertsen, Dorit Skov, Karin Larsen, Marit Pedholt, Pi Ørum, Annette Moesby Nielsen, and Tom Halborg for their assistance in this project.

References

Amrick, C.L. and D.A. Bennett, 1987, *N*-Methyl-D-aspartate produces discriminative stimuli in rats, Life Sci. 40, 585.

Arnt, J., P. Krogsgaard-Larsen and U. Madsen, 1992, Differential discriminative stimulus properties of NMDA and ATPA, an AMPA receptor agonist, J. Psychopharmacol. A63.

Bisaga, A., P. Krzascik, E. Jankowska, W. Palejko, W. Kostowski and W. Danysz, 1993, Effect of glutamate receptor antagonists on *N*-methyl-D-aspartate-and (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-induced convulsant effects in mice and rats, Eur. J. Pharmacol. 242, 213.

Brehm, L., F.S. Jørgensen, J.J. Hansen and P. Krogsgaard-Larsen, 1988, Agonists and antagonists for central glutamic acid receptors, Drug News Perspect. 1, 138.