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European Journal of Pharmacology 338 (1997) 233–242

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Novel NMDA/glycine site antagonists attenuate cocaine-induced behavioral toxicity

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Received 1 May 1997; revised 9 September 1997; accepted 19 September 1997

Abstract

N-Methyl-D-aspartate (NMDA)/glycine site antagonists were tested for their ability to prevent cocaine-induced convulsions and lethality in Swiss Webster mice. Pre-treatment of mice with the novel NMDA/glycine site antagonists ACEA-1021 (5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione) or ACEA-1328 (5-nitro-6,7-dimethyl-1,4-dihydro-2,3-quinoxalinedione) attenuated cocaine-induced convulsions; these effects were pharmacologically antagonized with D-cycloserine. The structurally-related NMDA/glycine site antagonist DCQX (6,7-dichloroquinoxaline-2,3-dione) and the structurally-unrelated NMDA/glycine site partial agonist HA-966 (3-amino-1-hydroxy-2-pyrrolidinone) also attenuated cocaine-induced convulsions, with the *R*(+)-isomer of HA-966 being more effective than the *S*(−)-isomer. In contrast, the selective α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptor antagonist, NBQX (1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide), failed to provide statistically significant protection although it shares the 2,3-quinoxalinedione structure of DCQX and the ACEA compounds. Pre-treatment with ACEA-1021, ACEA-1328, DCQX, or *R*(+)-HA-966 also attenuated cocaine-induced lethality in mice. Significantly, post-treatment with ACEA-1021, immediately prior to or after the onset of seizures, prevented death in up to 86% of mice receiving a lethal dose of cocaine; post-treatment with vehicle resulted in death of all mice. The results suggest the utility of targeting excitatory mechanisms for the treatment of cocaine overdose and offer a novel base structure from which effective pharmacotherapies can be developed. © 1997 Elsevier Science B.V.

Keywords: Cocaine; Convulsion; Lethality; NMDA receptor; Toxicity

1. Introduction

Cocaine has been reported to be the third most commonly abused drug, after alcohol and marijuana (Johnson and Vocci, 1993). It is responsible for more serious intoxications and deaths than any other illicit drug (Benowitz, 1993). No pharmacotherapies are currently available to effectively treat cocaine overdoses, nor do we have much insight into the mechanism(s) underlying its toxic effects (Johnson and Vocci, 1993). The acute toxic effects of cocaine overdose include convulsions, respiratory arrest, cardiovascular collapse and death (Benowitz, 1993). Since there is evidence for the involvement of *N*-methyl-D-aspartate (NMDA) receptors in convulsions, respiratory rhythms and cardiovascular control (Collingridge and Watkins, 1994; Meldrum, 1994; West and Huang, 1994; Berger et al., 1995), systems that are all seriously compromised after an overdose of cocaine, our working hypothesis is that modulation of activity at NMDA receptors can attenuate the cascade of secondary events that accompany a cocaine overdose.

Recently, a series of quinoxaline-2,3-dione derivatives (referred to as the ACEA compounds) have been synthesized and pharmacologically characterized (Keana et al., 1995; Woodward et al., 1995a,b). These novel ligands bind to the glycine site of NMDA receptors with nanomolar affinities and show relatively little affinity for non-NMDA (AMPA/kainate) receptors (Woodward et al., 1995a). They further exhibit up to a 50-fold variation in sensitivity, depending on NMDA receptor subunit composition (NR1A, NR2A-2D) (Woodward et al., 1995a). These compounds possess an in vivo pharmacological profile indicative of

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functional NMDA receptor antagonism and they have excellent systemic bioavailability. Pharmacological characterizations show that these compounds are effective neuroprotectants in a rat focal ischemic model of stroke (Warner et al., 1995), attenuate a cardiac arrest-induced movement disorder in rats (Matsumoto et al., 1995), serve as neuroprotectants in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of Parkinson's disease (Kanthasamy et al., 1996), attenuate nociceptive responses in mice (Vaccarino et al., 1993; Lutfy and Weber, 1996) and inhibit the development of opioid tolerance in mouse models of antinociception (Lutfy et al., 1995, 1996). In addition to being well tolerated in vivo, previous studies have shown that these NMDA/glycine site antagonists do not appear to substitute for phencyclidine (PCP) in drug discrimination studies (Balster et al., 1995) and lack overt PCP-like side effects such as barrel rolling, hyperactivity and ataxia which have been a major concern with other types of NMDA receptor antagonists (Koek and Colpaert, 1990; Carter, 1994). Another advantage of NMDA/glycine site antagonists is that they do not appear to induce neuronal vacuolation which is an unfavorable side effect of many competitive and noncompetitive NMDA receptor antagonists (Hargreaves et al., 1993; Auer, 1997; Hawkinson et al., 1997).

Although the number of available NMDA/glycine site antagonists for in vivo studies has been limited in the past, these novel quinoxaline-2,3-diones offer a new base structure for the development of effective therapeutics for the treatment of cocaine abuse and toxicity. Therefore, two of these novel quinoxalinedione derivatives, ACEA-1021 and ACEA-1328, were tested for their ability to block cocaine-induced convulsions and lethality in mice. As a positive control, the $R(+)$- and $S(-)$-isomers of the structurally-unrelated NMDA/glycine site partial agonist HA-966 were tested (Singh et al., 1990). As a negative control, the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor preferring antagonist, NBQX, a 2,3-quinoxalinedione that is structurally similar to the ACEA compounds, was also tested (Sheardown et al., 1990).

2. Materials and methods

2.1. Animals

Male Swiss Webster mice (22–32 g) were used (Simonsen, Gilroy, CA, USA; Harlan, Indianapolis, IN, USA; Charles River, Portage, MI, USA). The mice were housed in groups of 4–6 with a 12:12 h light/dark cycle and ad libitum food and water. All procedures were performed as approved by the Institutional Animal Care and Use Committees at the University of California Irvine and the University of Oklahoma Health Sciences Center.

2.2. Drugs

Cocaine hydrochloride and D-cycloserine were obtained from Sigma (St. Louis, MO, USA). $R(+)$-3-Amino-1-hydroxy-2-pyrrolidinone [$R(+)$-HA-966], $S(-)$-3-amino-1-hydroxy-2-pyrrolidinone [$S(-)$-HA-966], 6,7-dichloroquinoxaline-2,3-dione (DCQX) and 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX) were purchased from Research Biochemicals International (Natick, MA, USA). ACEA-1021 (5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione) and ACEA-1328 (5-nitro-6,7-dimethyl-1,4-dihydro-2,3-quinoxalinedione) were obtained from ACEA Pharmaceuticals/CoCensys (Irvine, CA, USA). The quinoxalinediones were dissolved in 50% dimethylsulfoxide (DMSO) (Sigma). Cocaine, $R(+)$-HA-966 and $S(-)$-HA-966 were dissolved in sterile saline (Abbott Laboratories, North Chicago, IL, USA).

2.3. Convulsions

Convulsions were induced in mice with cocaine (60 mg/kg, i.p.). Convulsions were operationally defined as the loss of righting reflexes for at least 5 s and the occurrence of clonic limb movements (Witkin and Tortella, 1991); this dose of cocaine produced convulsions in 100% of our animals. Mice were injected (i.p.) 15 min before administration of a convulsive dose of cocaine with one of the following pre-treatments: ACEA-1021 (30, 50, 60 mg/kg, $n = 28$), ACEA-1328 (10, 15, 20 mg/kg, $n = 31$), DCQX (15, 30, 60, 90 mg/kg, $n = 35$), $R(+)$-HA-966 (5, 10 mg/kg, $n = 20$), $S(-)$-HA-966 (5, 10 mg/kg, $n = 20$), saline ($n = 5$), or 50% DMSO ($n = 4$). NBQX (40, 80 mg/kg, $n = 14$) was administered 3–5 min before cocaine because previous studies report that it has short-lived effects (Taylor and Vartanian, 1992). Following injection with cocaine, the animals were observed for 30 min for the occurrence of a seizure.

To further evaluate the involvement of the glycine modulatory site on the NMDA receptor in the protective effects of the antagonists, D-cycloserine was used to pharmacologically antagonize the anticonvulsive effects of ACEA-1021 and ACEA-1328. Previous reports demonstrate that D-cycloserine is a partial agonist at the glycine modulatory site; it lacks interactions with the inhibitory (strychnine-sensitive) glycine receptor; it can attenuate antagonist responses mediated through the NMDA/glycine modulatory site; and it has the ability to cross the blood–brain barrier (Watson et al., 1990; Wood, 1995). Therefore, D-cycloserine (10 mg/kg, i.p.) and one of the antagonists (ACEA-1021 60 mg/kg, i.p., $n = 7$; ACEA-1328 20 mg/kg, i.p., $n = 5$) or vehicle ($n = 5$) were administered, followed 15 min later with a convulsive dose of cocaine (60 mg/kg, i.p.). After receiving cocaine, the mice were observed for 30 min for the occurrence of convulsions.

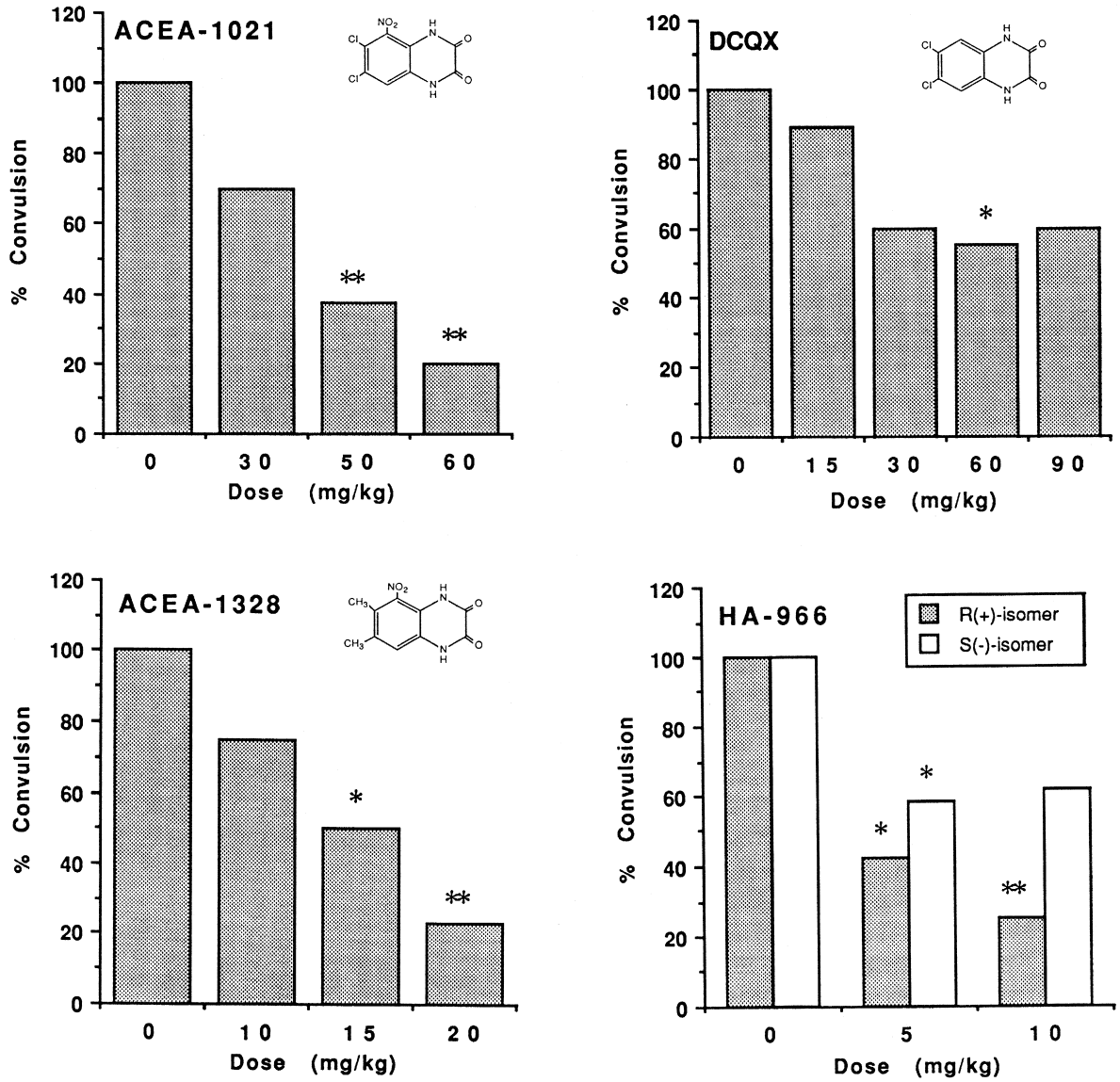

Fig. 1. NMDA/glycine site antagonists attenuate cocaine-induced convulsions. Mice were pre-treated (i.p.) with one of the following NMDA/glycine site antagonists: ACEA-1021 (0–60 mg/kg), ACEA-1328 (0–20 mg/kg), DCQX (0–90 mg/kg), or HA-966 (0–10 mg/kg). After 15 min, they were injected with a convulsive dose of cocaine (60 mg/kg, i.p.). The quinoxalinediones and the structurally-unrelated HA-966 compounds attenuated convulsions in a dose-dependent manner. $^{*}P < 0.05$ and $^{**}P < 0.01$ as compared to the vehicle control.

2.4. Lethality

Mice were injected (i.p.) 15 min before administration of cocaine (125 mg/kg, i.p.) with one of the following pre-treatments: ACEA-1021 (30, 40, 50, 70 mg/kg, $n = 36$), ACEA-1328 (10, 20, 30 mg/kg, $n = 24$), DCQX (30, 60, 90 mg/kg, $n = 15$), $R(+)$-HA-966 (1, 2.5, 5, 10 mg/kg, $n = 18$), saline ($n = 5$), or 50% DMSO ($n = 13$). Since under the pre-treatment condition, ACEA-1021 was the most effective quinoxalinedione, it was further evaluated after post-treatment. In the post-treatment condition, mice were injected with cocaine (125 mg/kg, i.p.) to induce lethality. ACEA-1021 (40 mg/kg, i.p.) was then administered either immediately after the first seizure ($n = 7$) or immediately preceding it (i.e. the mice were hyperactive and falling over, but not yet convulsing, $n = 7$). Post-treatment with vehicle ($n = 8$) served as the control. Death within 30 min of the cocaine injections was the behavioral endpoint. Those animals surviving the 30 min testing session were checked again after 24 h to assess the long term effects of the protection.

Since most cocaine overdoses occur in people who have been previously exposed to cocaine (Benowitz, 1993), another group of mice ($n = 25$) were injected once a day for 7 days with a moderate, subconvulsive dose of cocaine (40 mg/kg, i.p.). Two hours following the last exposure to cocaine, the mice were overdosed with 125 mg/kg of cocaine. After the onset of convulsions, the mice were

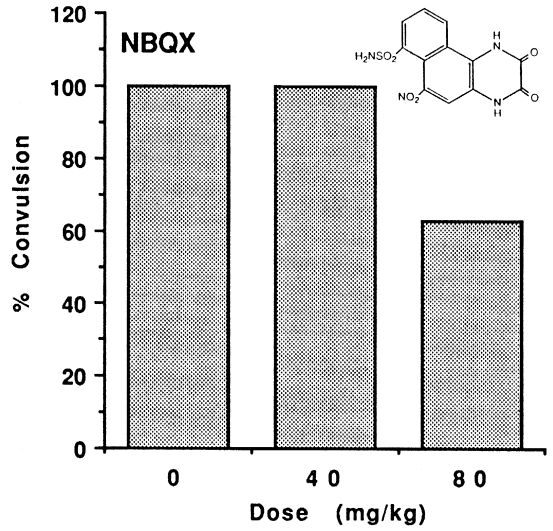

Fig. 2. The selective AMPA receptor antagonist, NBQX, failed to provide statistically significant protection against cocaine-induced convulsions. Mice were pre-treated for 3–5 min with NBQX (40 or 80 mg/kg, i.p.), followed by a convulsive dose of cocaine (60 mg/kg, i.p.). NBQX possesses a quinoxalinedione structure similar to the ACEA compounds, but it has relatively little affinity for NMDA/glycine sites.

post-treated (i.p.) with either vehicle ($n = 9$) or ACEA-1021 (40 mg/kg, $n = 16$). The percentage of dead animals 30 min and 24 h after the overdose were noted.

2.5. Statistics

The data were analyzed with Fisher's exact tests or *t*-tests (GraphPad InStat, San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Convulsions

Intraperitoneal injections of 60 mg/kg cocaine produced a reliable loss of righting reflexes in mice within 3–5 min, followed by clonic seizures. Pre-treatment of mice with the NMDA/glycine site antagonists, ACEA-1021, ACEA-1328, DCQX, *R*(+)-HA-966 or *S*(−)-HA-966 15 min prior to cocaine significantly attenuated cocaine-induced convulsions ($P < 0.05$ for at least one dose of each drug; Fig. 1). In terms of the enantioselectivity of the isomers of HA-966, *R*(+)- was more effective than *S*(−)-; although this difference was not statistically significant, the pattern is consistent with a mechanism involving the glycine modulatory site. None of the vehicles (saline, 50% DMSO) had protective effects on the convulsions. In addition, the structurally-related 2,3-quinoxalinedione, NBQX, which lacks significant interactions with the glycine site on the NMDA receptor, failed to provide statistically significant protection against the convulsions (Fig. 2). Further confirming the involvement of the NMDA/glycine site in the protective effects is the observation that the anticonvulsive effects produced by ACEA-1021 and ACEA-1328 were pharmacologically antagonized with the NMDA/glycine site agonist D-cycloserine ($P < 0.01$); D-cycloserine alone had no protective effects (Fig. 3).

3.2. Lethality

Pre-treatment of mice with the NMDA/glycine site antagonists, ACEA-1021, ACEA-1328, DCQX, or *R*(+)-

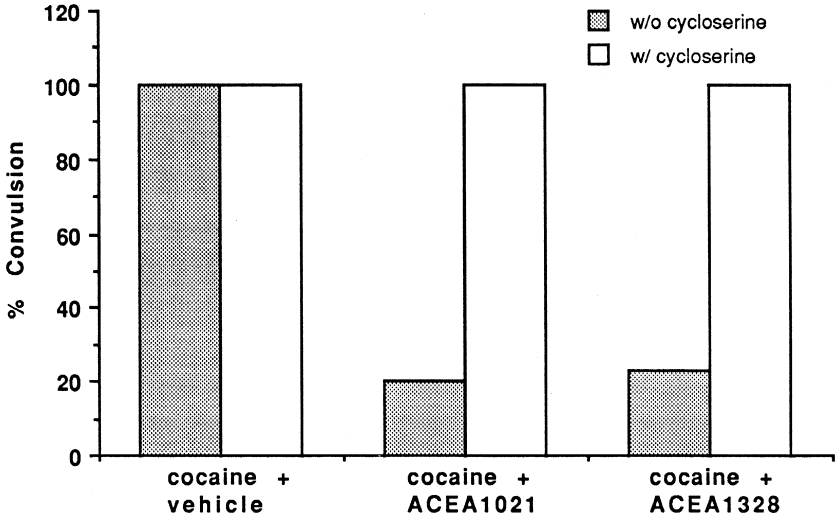

Fig. 3. Functional antagonism of the anticonvulsive effects of the ACEA compounds with D-cycloserine. Mice in the 'with cycloserine' group were co-treated (i.p.) with the NMDA/glycine site agonist, D-Cycloserine (10 mg/kg) and an ACEA compound (ACEA-1021, 60 mg/kg; ACEA-1328, 20 mg/kg) or vehicle during the pre-treatment period. Mice in the 'without cycloserine' group, received antagonist or vehicle alone during the pre-treatment period. All of the animals were injected with cocaine (60 mg/kg, i.p.) 15 min after the pre-treatment and were observed for the next 30 min for the onset of convulsions. D-Cycloserine totally reversed the protection provided by the ACEA compounds ($P < 0.01$), further implicating the NMDA/glycine site in the effects.

HA-966 15 min prior to injection of cocaine attenuated cocaine-induced lethality as compared to animals pre-treated with vehicle (Fig. 4). The protection provided by the most effective doses of ACEA-1021 and *R*(+)-HA-966 were statistically significant ($P < 0.05$), while the attenuation of lethality by ACEA-1328 and DCQX approached statistical significance, but fell just short of it. When higher doses of ACEA-1021, ACEA-1328, DCQX and *R*(+)-HA-966 were used as the pre-treatment, the antagonists were much less effective in protecting the animals from death. When combined with cocaine: 70 mg/kg of ACEA-1021 resulted in lethality in 75% of animals; 30 mg/kg of ACEA-1328 or 10 mg/kg of *R*(+)-HA-966 resulted in death of 80% of mice; 90 mg/kg of DCQX produced death in 100% of animals.

The average sequence of events after a lethal injection of cocaine (125 mg/kg) was the occurrence of seizures after 2.15 min, ultimately followed by death 4.44 min after the injection of cocaine. Following a lethal injection of cocaine, post-treatment with ACEA-1021 immediately after the first seizure resulted in survival of 57% of animals ($P < 0.05$); post-treatment with ACEA-1021 immediately before the first seizure increased survival to 86% ($P < 0.01$; Fig. 5). The protection was, for the most part, maintained after 24 h (Fig. 5) since there was no significant difference between the data obtained 30 min after the overdose versus 24 h later (Fisher's exact test, n.s.). In contrast to the effectiveness of ACEA-1021, post-treatment with vehicle did not provide protection. It should also be noted that the overall behavior and health of the surviving animals were

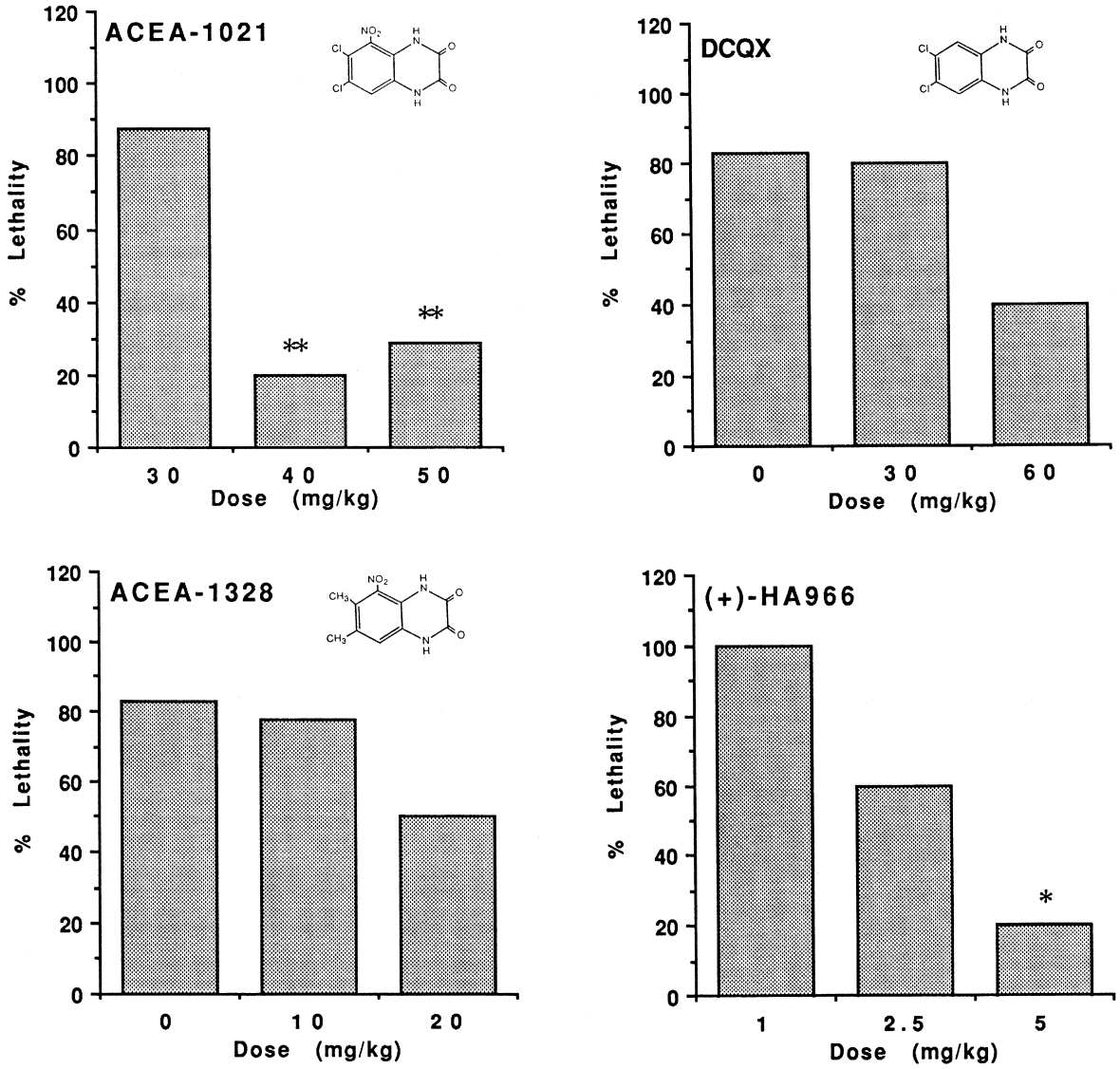

Fig. 4. Pre-treatment with NMDA/glycine site antagonists attenuates cocaine-induced lethality. Mice were pre-treated (i.p.) with one of the following NMDA/glycine site antagonists: ACEA-1021 (0–50 mg/kg), ACEA-1328 (0–20 mg/kg), DCQX (0–60 mg/kg), or *R*(+)-HA-966 (1–5 mg/kg). After 15 min, they were injected with cocaine (125 mg/kg, i.p.) and death within 30 min was the behavioral endpoint. $^{*}P < 0.05$ and $^{**}P < 0.01$ as compared to pre-treatment with vehicle.

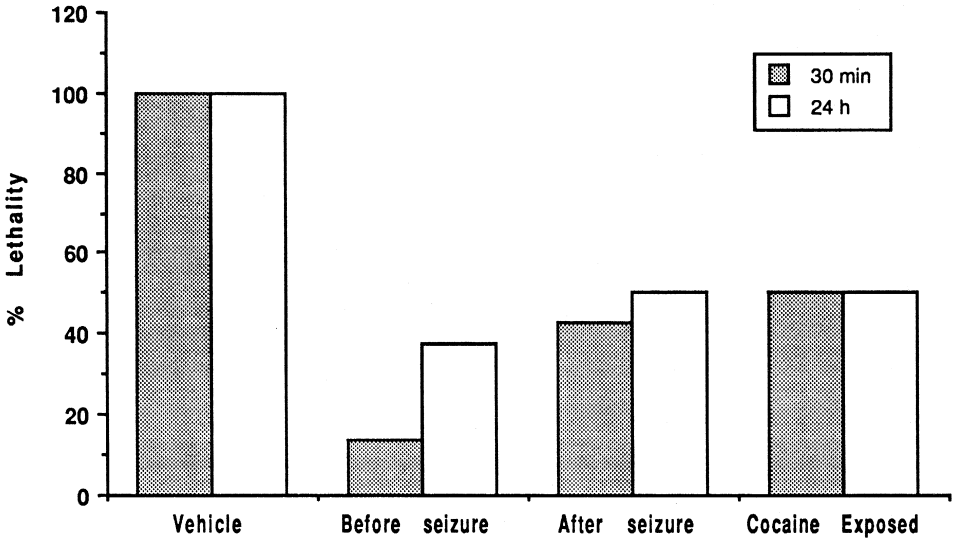

Fig. 5. Post-treatment with ACEA-1021 attenuates cocaine-induced lethality. All of the mice were injected with a lethal dose of cocaine (125 mg/kg, i.p.). Death after 30 min and 24 h of the overdose was the behavioral endpoint. ACEA-1021 (40 mg/kg, i.p.) rescued a significant proportion of mice from death ($P < 0.05$) when it was administered either immediately before or after the onset of the first convulsion in naive mice (middle bars labeled 'before seizure' and 'after seizure'). Post-treatment with ACEA-1021 (40 mg/kg, i.p.) after the onset of convulsions also provided significant protection in non-kindled mice who were exposed to cocaine (40 mg/kg/day) for 7 days prior to receiving an overdose of cocaine ('cocaine exposed,' $P < 0.05$). Post-treatment with vehicle in both naive and cocaine exposed mice resulted in the death of all animals; therefore, although the vehicle controls for the two groups of mice were run separately, they are depicted in the figure as a single set of bars (first set of bars).

qualitatively better after the post-treatment condition, as compared to pre-treatment.

Mice that were pre-exposed to a moderate, subconvulsive dose of cocaine for 7 days were divided into two post-treatment groups based on their responses to cocaine during this initial exposure period (kindled versus non-kindled). Kindled mice ($n = 6$) exhibited progressive and significant weight loss during the 7 day exposure period, which was accompanied by the onset of seizures following administration of a previously subconvulsive dose of cocaine. Mice in the kindled group lost on average 3 ± 1 g of weight during the 7 day exposure period to cocaine (weight on day 1–day 7) and exhibited seizures on at least two consecutive days during this time. Two additional mice that exhibited kindling ultimately died by the end of the 7 day exposure period to cocaine and did not undergo post-treatment with antagonist. This pattern is similar to that observed by others (Itzhak and Stein, 1992; Shimosato et al., 1996). However, in our hands, most mice from this outbred strain ($n = 19$) exhibited no obvious signs of kindling during the 7 day exposure period to a subconvulsive dose of cocaine. The vast majority of mice in the non-kindled group remained seizure free, although 15% of the mice exhibited convulsions on one day during the 7 day exposure period. Further, the non-kindled mice maintained their body weights, and as a group there was less than a half gram change in body weight. Qualitatively, the appearance of the non-kindled mice were similar to that of drug-free animals whereas the kindled mice looked unwell (e.g. poor grooming, sunken eyes, noticeable weight loss). Since there appeared to be a difference in the overall health of kindled versus non-kindled animals, the effects of the post-treatment with antagonist were analyzed separately in these two groups of mice.

Following the overdose of cocaine, there was a significant difference in the ability of ACEA-1021 to rescue kindled versus non-kindled mice. Non-kindled mice were protected from death if post-treated with ACEA-1021 ($P < 0.05$), but kindled mice could not be rescued after an overdose of cocaine. The convulsions exhibited by non-kindled mice pre-exposed to cocaine were much more severe than the convulsions seen in naive mice, but the protection provided by ACEA-1021 remained intact and was maintained for up to 24 h (Fig. 5). Further, non-kindled mice who were post-treated with ACEA-1021 but ultimately died experienced a noticeable, but not statistically significant, increase in survival time (9.48 ± 2.37 min). Similarly, although 100% of the kindled mice ultimately died within 30 min of receiving a lethal of cocaine, they survived for twice as long when post-treated with ACEA-1021 (9.88 ± 1.96 min); this protection, although noticeable, was not statistically significant.

4. Discussion

The novel, NMDA/glycine site antagonists ACEA-1021 and ACEA-1328 were effective in blocking cocaine-induced convulsions and lethality. Pharmacological characterizations using receptor binding and patch clamp studies show that these ligands have nanomolar affinities for the glycine site of NMDA receptors (Woodward et al., 1995a).

Their protective actions appear to be mediated through the glycine modulatory site of NMDA receptors rather than some unidentified property associated with the chemical structure of the compounds because the structurally unrelated NMDA/glycine site partial agonist HA-966 was also effective in attenuating cocaine-induced convulsions. Further, the *R*(+)-isomer of HA-966 was more effective than the *S*(−)-isomer, a pattern that is consistent with effects mediated through the glycine modulatory site (Singh et al., 1990). In addition, the NMDA/glycine modulatory site agonist D-cycloserine, pharmacologically antagonized the anticonvulsive effects of ACEA-1021 and ACEA-1328. Further supporting the importance of the NMDA/glycine site in the protective actions is the observation that the AMPA receptor-selective quinoxalinedione, NBQX, which lacks activity at the NMDA/glycine site, fails to provide statistically significant protection against the toxic effects of cocaine even though it shares a similar chemical structure to the ACEA compounds. NBQX is known to cross the blood–brain barrier and the specific pre-treatment conditions and doses of NBQX were chosen because they were expected to be active based on previous reports of the effectiveness of NBQX in protecting against other types of convulsions in animals (Turski et al., 1992). For example, NBQX has been reported to antagonize audiogenic seizures, pentylenetetrazol-induced kindling, and convulsions induced with picrotoxin, pentylenetetrazol, 3-mercaptoproprionate, and methyl-4-ethyl-6,7-dimethoxy-9H-pyrido-(3,4-b)-indole-3-caboxylate with ED_{50} values of 40 mg/kg or less (Smith et al., 1991; Turski et al., 1992; Shimizu-Sasamata et al., 1996). Furthermore, NBQX has been reported to be effective against other pathological conditions (e.g. ischemia, spasticity, neurodegeneration) at even lower doses (Sheardown et al., 1990; Turski et al., 1992; Lallement et al., 1993; Shimizu-Sasamata et al., 1996). Therefore, the present finding that the 40 mg/kg dose of NBQX was totally ineffective against cocaine-induced convulsions, and that the 80 mg/kg dose produced marginal protection in less than half of the animals must be considered in light of the ability of this drug to produce dramatic changes in other pathological states. It should also be noted that at these high doses, NBQX is known to produce locomotor depression and some ataxia (cf. Zarnowski et al., 1993; Löscher and Hönack, 1994). Therefore, a comparison of the results with NBQX with those of the ACEA ligands suggest that simply having the 2,3-quinoxalinedione structure is not sufficient for producing protective effects against cocaine, and that the ability to antagonize the glycine modulatory site of the NMDA receptor is a key feature that affords protection.

It should be noted that this is not the first time that NMDA receptor antagonists have been reported to protect against cocaine-induced convulsions. The non-competitive NMDA receptor antagonist, MK-801, has been shown to be effective in preventing cocaine-induced convulsions (Derlet and Albertson, 1990). However, MK-801 cannot prevent death and it also causes severe side effects such as ataxia, headweaving, and hyperactivity, which greatly limit any potential therapeutic benefit (Witkin and Tortella, 1991). In a more promising set of studies, the NMDA/glycine site modulators 7-chlorokynurinic acid and ACPC (1-amino-1-cyclopropane-carboxylic acid) were shown to dose-dependently attenuate cocaine-induced convulsions without themselves causing behavioral neurotoxic effects (Witkin and Tortella, 1991). The results presented herein using novel ligands with improved systemic bioavailability, confirm the effectiveness of targeting the glycine modulatory site of the NMDA receptor.

In contrast with the disappointing effects of many other classes of compounds in protecting against cocaine-induced lethality, the NMDA/glycine site antagonists were particularly impressive in this regard. Pre-treatment with ACEA-1021 or *R*(+)-HA-966 produced statistically significant protection from the lethal effects of cocaine. Although the effects of ACEA-1328 and DCQX were not quite statistically significant, both ligands attenuated lethality when given before an overdose of cocaine. ACEA-1021, which was the most effective NMDA/glycine site antagonist in the pre-treament condition, also prevented death in a significant proportion of animals when given after the overdose of cocaine. Over half of the animals survived when ACEA-1021 was administered after the occurrence of seizures and all but one animal lived when the antagonist was administered just prior to the start of the seizures. Post-treatment with ACEA-1021 also rescued from death animals that had been pre-exposed to cocaine prior to receiving an overdose. This protection under the post-treatment condition is particularly impressive because in our animals, death typically occurs within a few minutes of the first seizure and in animals pre-exposed to cocaine, the convulsions were particularly severe. Further, even without additional supportive therapy after post-treatment with ACEA-1021, the survival rate was for the most part, maintained after 24 h. To our knowledge, no other compound is as effective in combating cocaine-induced lethality.

The NMDA/glycine site antagonists that were used in this study appear to be much improved in terms of toxic risks, as compared to earlier generations of antagonists. Within the dose ranges used in this study, the only side effects that were observed were sedation and hypothermia at higher doses. The ataxia, headweaving and hyperactive behaviors that have been problematic with MK-801 were absent. Further, preliminary neurohistopathological examination of Nissl stained brain sections from mice treated with ACEA-1021 who survived the overdose revealed no obvious loss of hippocampal neurons. This is significant because many types of seizures cause excitotoxic damage to hippocampal neurons and mice who experience a cocaine overdose convulse (Olney et al., 1986; Cavazos et al., 1994; Savolainen et al., 1994; Wasterlain and Shirasaka, 1994; Bitner et al., 1995). Together with reports

from other labs that ACEA-1021 and *R*(+)-HA-966 themselves do not produce neuronal necrosis and vacuolation under conditions where such changes are induced by many other competitive and noncompetitive NMDA receptor antagonists (Hargreaves et al., 1993; Auer, 1997; Hawkinson et al., 1997), the data demonstrate that these new compounds have a much improved therapeutic profile as compared to earlier generations of antagonists.

Although sedation and hypothermia can be produced by higher doses of the NMDA/glycine site antagonists (Weber, personal communication), the protective effects cannot be attributed solely to these factors. Although we did not quantitatively measure sedation and hypothermia in these experiments, these effects were qualitatively noticeable (by observation or touch) when higher doses of the antagonists were used. However, it should be noted that while the sedative and hypothermic effects of the antagonists were most obvious at the highest doses of antagonists tested (30 mg/kg for ACEA-1328 and 70 mg/kg for ACEA-1021), at these doses, the antagonists failed to provide protection, suggesting that the dose curve for protection is in the form of an inverted U, while the dose curves for sedation and hypothermia are still in the linear range at these higher doses. If the mechanisms underlying sedation and hypothermia versus protection from cocaine-induced behavioral toxicity were shared, the dose curves should be parallel. Since this was not observed, the protection against cocaine-induced seizures and lethality cannot be accounted for by the sedative and hypothermic effects of the antagonists. Nevertheless, the ability of the antagonists to produce these physiological changes should only optimize their clinical potential because these alterations appear to be positive clinical indices in terms protecting against other types of cerebral insults and in facilitating the clinical management of overdose patients who can otherwise be disruptive (Wetli and Fishbain, 1985; Dietrich, 1992).

In terms of the U-shaped dose–response curves for the NMDA/glycine site antagonists, several possibilities may account for this pattern. First, as higher doses of the antagonists are used, they may become somewhat nonselective for NMDA receptors and alter other neurochemical systems that may contribute to the toxic effects of cocaine. Second, the compounds may actually be partial agonists. We know that this is true of HA-966 and as a weak partial agonist, the U-shaped dose curve for HA966 would be consistent with the fact that at higher doses, HA-966 would act as a NMDA/glycine site agonist, and thus expected to worsen the toxicity. At this time, however, there is no definitive proof for either of these possibilities, and it is worth remembering that most drugs, even those that are generally considered to be safe, can produce toxicity and/or loss of efficacy at high enough doses. Therefore, the U-shaped dose curves of the NMDA/glycine site antagonists are not unique in this regard.

The characteristic of these antagonists that make them unique, however, is their ability to dramatically antagonize cocaine-induced behavioral toxicity under rigorous in vivo conditions. The compounds represent the first of their kind to effectively block the convulsions and lethality produced by a cocaine overdose. Under the conditions in which the experiments were conducted and those that exist in the clinic, if left untreated or treated with existing pharmacotherapies, the lethality rate is virtually 100%. Therefore, although the estimated margin of safety of the NMDA/glycine site antagonists in an overdose situation is not great, their potential therapeutic benefit is considerable. The best compound that we tested, ACEA-1021, could prevent lethality under conditions where death typically occurs within a few minutes of the overdose. There is no other compound, to our knowledge, that can work this quickly and effectively.

One potential strategy to widen the therapeutic window of the ACEA compounds and minimize potential side effects involves combining an ACEA ligand with NBQX. Although NBQX itself did not statistically significantly alter the behavioral toxicity produced by cocaine, low doses of NBQX have been reported by others to potentiate the activity of other anticonvulsants and neuroprotectants (Zarnowski et al., 1993; Lippert et al., 1994). Further studies are therefore underway to determine whether NBQX can be used in combination with NMDA/glycine site antagonists to improve their margin of safety.

In sum, these novel quinoxalinediones are the first of their kind in showing protective effects against cocaine-induced convulsions and lethality. They appear to produce their protection by antagonizing NMDA/glycine sites, suggesting the involvement of excitatory mechanisms in cocaine-induced toxicity. These compounds offer greatly improved systemic bioavailablity and reduced toxicity as compared to other NMDA receptor antagonists. Although the estimated margin of safety of these antagonists can be improved upon, the existing compounds are effective and provide a novel base structure from which better pharmacotherapies can be developed to treat overdose situations and prevent cocaine-related fatalities.

Acknowledgements

We thank Dr. Roger Hornbrook for the gift of cocaine (University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA) and Dr. Eckard Weber and Dr. Richard Carter for providing ACEA-1021 and ACEA-1328 (ACEA Pharmaceuticals/CoCensys, Irvine, CA, USA). We also appreciate the technical assistance of Eddie Ramirez (University of California Irvine, Irvine, CA, USA) and Michele Friedman, Joshua Blyden and Michelle Brown (University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA). The studies described herein were supported in part by an OCAST award for project #H97-

057, from the Oklahoma Center for the Advancement of Science and Technology (R.R.M.) and a Summer Undergraduate Research Experience fellowship from the University of Oklahoma Health Sciences Center (R.L.B.).

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