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Impairment of contextual fear conditioning in rats by Group I mGluRs: Reversal by the nootropic nefiracetam

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Abstract

The blockade of Group I metabotropic glutamate receptors (mGluRs) may be a potential strategy for prevention therapy of neurotoxicity. We here confirm previous reports that systemic application of the Group I antagonist, 1-aminoindan-1,5-dicarboxylic acid (AIDA), causes amnesia in a contextual fear conditioning paradigm in rats. This deficit was fully reversed by long-term pretreatment with the nootropic nefiracetam, which in fact obtained supranormal performance. Our data suggest that application of Group I antagonists to prevent neurotoxicity, combined with nootropic treatment to prevent cognitive deficits, may be a therapeutic strategy for the development of novel antineurotoxic treatments.

Keywords: Metabotropic glutamate receptors (mGluRs); Nootropic; Fear conditioning; Memory; Rat

1. Introduction

Glutamate, the major excitatory neurotransmitter in the central nervous system, has multiple functions and is involved in both normal and abnormal functioning of neurones. These functions may engage both ionotropic and metabotropic glutamate receptors (mGluRs), and the latter have recently been the subject of intense investigations as targets for potentially clinically approvable drugs in the treatment of neurotoxicity.

Cloned mGluRs are currently categorised in three groups acting on either phospholipase C (Group I: mGluR1 and 5) or adenylate cyclase (Group II: mGluR2 and 3; Group III: mGluR4, 6, 7 and 8) (Jane and Doherty, 2000). Activation of Groups II and III mGluRs has been proven to be neuroprotective (Bruno et al., 1995, 1997, 1998; Buisson and Choi, 1995). For Group I mGluRs, recently designed selective agonists and antagonists have revealed that blockade of the receptors, and more specifically mGluR5, may be analgesic (Binns and Salt, 2001) and/or neuroprotective against glutamatergic hyperexcitation in culture and in vivo (Bruno et al., 1999, 2000, 2001; Strasser et al., 1998). Similar results were reported for experimentally induced hypoxia/hypoglycemia (Schröder et al., 1999; Bruno et al., 1999). Activation of Group I mGluRs significantly exacerbated epileptiform activity (Aronica et al., 1997; Merlin and Wong, 1997), further supporting the notion that drugs acting on Group I mGluRs may be beneficial in the treatment of neurodegenerative disorders (Nicoletti et al., 1996). One widely used Group I mGluR antagonist is 1-aminoindan-1,5-dicarboxylic acid (AIDA). AIDA reduced the toxicity and cell death of NMDA-induced neurotoxicity in cultured cortical cells (Buisson and Choi, 1995; Strasser et al., 1997), and delayed degeneration of neurones after experientially induced transient global ischemia in gerbils (Cozzi et al., 1997). Thus, AIDA and other Group I antagonists may prove useful in the development of new lead compounds for treatments of neural excitotoxicity.

Simultaneously, it is important to know the role of Group I mGluRs in normal brain functioning. There is now strong evidence for a role of mGluRs in various learning situations and memory processes (for review, see

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Riedel, 1996; Riedel et al., 1996) and more recent work has confirmed that Group I plays an important part in such processes. In rats, for example, pharmacological blockade of Group I mGluRs has been shown to impair memory formation on contextual fear conditioning (Nielsen et al., 1997; Christoffersen et al., 1999a,b), fear-potentiated startle (Schulz et al., 2001), spatial learning in the T-maze (Balschun and Wetzel, 1998), radial arm maze (Balschun et al., 1999), the three-panel runway (Kishi et al., 1998; Ohno and Watanabe, 1998) or a three-choice maze (Christoffersen et al., 1999a,b). Interestingly, when Group I antagonists such as AIDA and 4-carboxyphenylglycine (4-CPG) were injected prior to acquisition training, learning itself remained unchanged, but performance during the memory test was impaired. This would suggest a selective role of Group I mGluRs in memory consolidation or recall, a finding supported by studies using mGluR1 or mGluR5 knockout mice (Conquet et al., 1994; Aiba et al., 1994; Lu et al., 1997). It is also consistent with the finding that posttraining injections of the Group I agonist, (*S*)-3,5-DHPG, facilitated consolidation in a passive avoidance task (Zalewska-Winska and Wisniewski, 2000; Car et al., 2000). Moreover, the expression of hippocampal mGluR5 following contextual fear conditioning was increased in a region- and time-specific manner (Riedel et al., 2000). The possibility that activity of Group I mGluRs is essential during recall still remains unexplored. Recent work using a hippocampus-dependent inhibitory avoidance paradigm, in which the Group I/II antagonist (*R*,*S*)-α-methylcarboxyphenylglycine (MCPG) was administered, however, suggests that this is likely (Barros et al., 2000; Izquierdo et al., 2000; Szapiro et al., 2000).

Taken together with the toxicity data, these data suggest that targeting Group I mGluRs for clinically used drugs might lead to amnesia. As such, a side effect is unwanted; coadministration of memory-reinstating/enhancing compounds may be indicated. We explored this possibility by coapplication of the nootropic compound, nefiracetam (*N*-(2,6-dimethyl-phenyl)-2(2-oxo-1-pyrrolidinyl) acetamide (for review, see Yamada and Nabeshima, 1996; Nabeshima, 1994). Nefiracetam is well known to attenuate amnesia possibly through activation of cholinergic, GABAergic and/or monoaminergic transmitter systems (Watabe et al., 1993). Electrophysiologically, nefiracetam increased voltage-dependent N-type and L-type calcium channels, thus promoting neural transmission (Hiramatsu et al., 1997; Yoshii et al., 1997). The ways in which it affects glutamatergic transmission remain elusive, but it could be argued that while mGluRs are blocked, the unspecific and widespread enhancement of other transmitter systems might attenuate or abolish the adverse effects of the mGluR blocker. Such a treatment may be particularly interesting, as small increments in cholinergic, GABAergic or monoaminergic tone are unlikely to be neurotoxic.

2. Materials and methods

2.1. Subjects

Male Lister hooded rats weighing 275–300 g at the start of the experiments and purchased from a commercial dealer (Harlan, Bicester, UK) were used. They were housed individually in climatized rooms at a fixed temperature (21 ± 2 °C) with food and water ad libitum and a 12:12 h light:dark cycle. Animals were randomly assigned to groups for the different experiments (see below) and were all naïve with respect to the task and drugs.

2.2. Drugs and drug application

AIDA (Tocris, Bristol, UK) was dissolved in equimolar NAOH and further diluted with physiological saline (0.9%). AIDA (0.18 mg/kg) or saline was injected intraperitoneally 25 min prior to behavioural testing in a volume of 0.5 ml/100 g body weight. This concentration had previously been determined as an effective dose in fear conditioning (Nielsen et al., 1997; Christoffersen et al., 1999a,b).

Nefiracetam (Daiichi Pharmaceutical, Tokyo, Japan) was dissolved in 0.5% carboxymethylcellulose (CMC) in various concentrations and administered orally in concentrations of 1, 3 or 10 mg/kg at a volume of 1 ml/100 g either acutely (Experiment 1a) 25 min prior to behavioural testing, or chronically starting 16 (Experiment 1b) or 7 (Experiment 2) days before habituation and throughout training. Chronically treated rats were given nefiracetam/CMC at the same time each day (5 p.m.). Piracetam (Sigma, Poole, UK) was also dissolved in CMC and administered orally at a dose of 500 mg/kg.

Groups and group sizes tested were:

Experiment 1a: effect of acute nefiracetam treatment on fear conditioning. (1) CMC ($n = 8$); (2) 1 mg/kg nefiracetam ($n = 8$); (3) 3 mg/kg nefiracetam ($n = 8$); (4) 10 mg/kg nefiracetam ($n = 8$).

Experiment 1b: effect of chronic nefiracetam treatment on fear conditioning. (1) CMC ($n = 8$); (2) 1 mg/kg nefiracetam ($n = 8$); (3) 3 mg/kg nefiracetam ($n = 8$); (4) 10 mg/kg nefiracetam ($n = 8$); (5) 500 mg/kg piracetam ($n = 8$).

Experiment 2: effect of chronic nefiracetam on AIDA-induced fear conditioning deficits. (1) CMC + saline ($n = 7$); (2) CMC + AIDA ($n = 8$); (3) 3 mg/kg nefiracetam + saline ($n = 15$); (4) 3 mg/kg nefiracetam + AIDA ($n = 12$).

2.3. Apparatus

Training and testing were conducted in a standard-sized conditioning box (Campden Instruments Loughborough, UK) placed in a sound-attenuating cubicle. The front door of the cubicle was open to enable video

observation and recording of the behaviour for subsequent scoring. A 3-W house light illuminated the conditioning box and a stainless steel grid floor was equipped for shock delivery through a scrambler. A fan provided constant background noise (65 dB) and an overhead loudspeaker delivered the tone (conditioning stimulus) of 10 kHz and 80 dB.

2.4. Experimental procedures

We conducted a series of experiments to establish the parameters to be used for combining drug application of nefiracetam and AIDA. Classical fear conditioning to context and tone was performed as described previously (Nielsen et al., 1997; Christoffersen et al., 1999b) with some slight modifications. Common to all experiments was that rats were habituated to the box for 20 min on Day 1. In Experiment 1A, we observed that the tone presented in Trial 1 of Day 2 before any conditioning took place caused substantial unconditioned fear responses due to its aversiveness. As a consequence, we modified the training protocol in Experiments 1B and 2 and introduced on Day 2 a session in which all animals were placed in the box and received an exposure to the high-pitch tone of 20 or 30 s, but no shock was delivered. After a further 30 s, animals were removed from the box. This served to reduce the unconditioned fear response. On the following days, training consisted of two trials per day with subjects placed in the box and allowed to explore for 2 min. Then, a period of 30 (Experiment 1) or 20 s (Experiment 2) was recorded as the pre-CS or context period (Phillips and LeDoux, 1992, 1995) and the next 30/20 s presented the CS with the tone being present. This was subsequently followed by a brief (500 ms) scrambled shock (unconditioned stimulus, US) of 0.2 (Experiment 1) or 0.25 mA (Experiment 2) intensity. After a 60-s intertrial interval, the pre-CS/CS/US sequence was repeated for Trial 2 and the session was terminated after a further 30 s. Animals returned to their home cages.

Freezing — defined as the absence of all movements except respiratory, combined with a typical crouching posture (Blanchard and Blanchard, 1969)—was recorded continuously during both pre-CS and CS using a stop watch by two experimenters blind to the experimental condition of the animal. Special weight is given to the performance during Trial 1 of each session since freezing in Trial 2 was confounded by the lingering effect of the US presented moments earlier during Trial 1.

In Experiment 1, we also recorded the activity of the animals in the conditioning box in order to establish that a change in freezing pattern due to drug exposure was not a result of hyper- or hypoactivity. The clear perspex front door of the conditioning box was therefore divided by a horizontal and a vertical line as suggested by Good and Honey (1997) so that horizontal and vertical crossings could be recorded. Vertical crossings consisted of full body movements across the midline, whereas horizontal movements corresponded to rearings. In the acute treatment (Experiment 1A), activity was recorded on the first training day during the initial 120 s prior to any CS and was thus not confounded by any fear conditioning. For the chronic nefiracetam protocol (Experiment 1B), we monitored activity during the initial 5 min of the habituation session.

3. Results

Previous work has repeatedly shown that AIDA blocks context, but not cue conditioning, in this fear-conditioning paradigm. Nothing, however, is known about nefiracetam and its potential to facilitate learning in this task. Moreover, we have found during several tests that animals readily achieve asymptotic freezing levels, at least during CS periods, when a 20-s CS and a shock intensity of >0.25 mA is applied. Under such conditions, it would be difficult to observe a learning enhancement. Weaker conditioning could be achieved by reducing the strength of the US (<0.25 mA) and/or prolonging the CS period to ≥ 30 s, and pilot data suggested that untreated control animals show freezing levels during pre-CS and CS periods, which were well below asymptotic levels (data not shown). This paradigm was used to establish the effective dose of nefiracetam for later administration to be applied in conjunction with AIDA (Experiment 2). Like other nootropic drugs, nefiracetam is much more potent when administered chronically for several days or weeks (Nabeshima, 1994; Yamada and Nabeshima, 1996). Thus, we compared acute and chronic treatments.

3.1. Experiment 1A: effect of acute nefiracetam treatment and fear conditioning

Nefiracetam in varying doses was administered orally 25 min prior to the training sessions. The results of this acute treatment are depicted in Fig. 1.

For the *context* period (Fig. 1A), freezing increased during Trials 3 and 5 in all groups, and this increase was more pronounced in the 3-mg/kg nefiracetam group compared to all other groups. A 4×3 factorial analysis of variance (ANOVA) with Drug (four doses) as between-subject and Trial (three levels) as within-subject factor revealed a main effect of Trial [$F(2,63)=5.5$, $P=.007$], no interaction ($F<1$) and a strong tendency for a Drug effect [$F(3,63)=2.5$, $P=.087$]. This would suggest that groups did not differ, but careful inspection of Fig. 1A tentatively indicates learning enhancement in the 3-mg/kg nefiracetam group. Interestingly, 3 mg/kg nefiracetam has been found to be the most potent dose to prevent beta-amyloid-induced memory deficits (Yamada et al., 1999). A planned two-way ANOVA comparing performance between CMC and 3 mg/kg nefiracetam animals confirmed such an enhancement with a main effect of Drug [$F(1,21)=10.3$, $P=.004$].

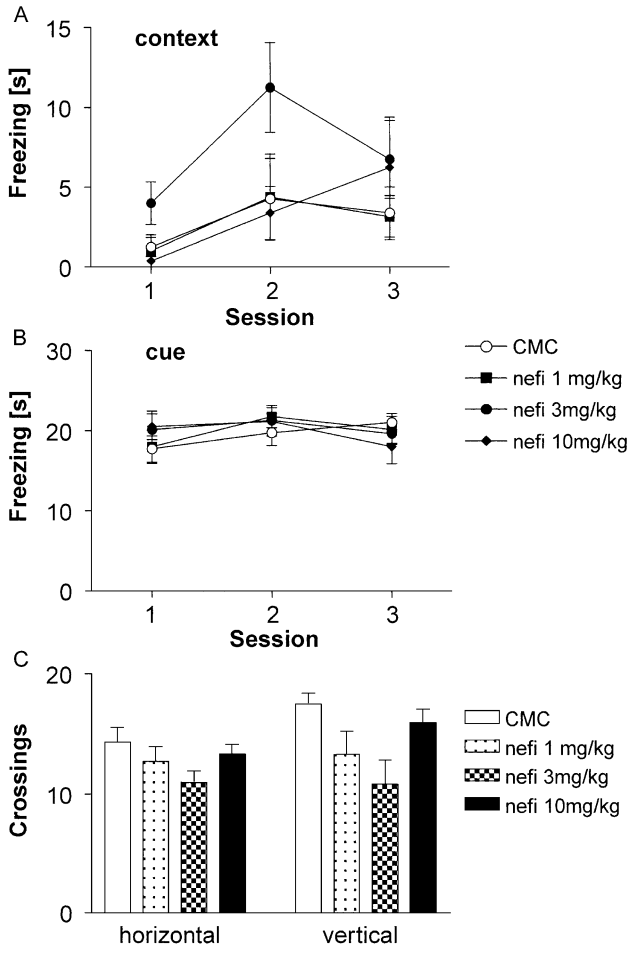

Fig. 1. Acute dose-dependent effects of nefiracetam on fear conditioning. Mean ± S.E.M. (A) Freezing to the context period (30 s) recorded during the first trial of each daily session. Vehicle (CMC) and three different concentrations of nefiracetam are shown. (B) Freezing to the cue (80 dB tone). (C) Activity measured as horizontal and vertical crossing 120 s prior to conditioning.

Freezing during the *tone* (CS) period (Fig. 1B) was similar in all groups with no change over the training period. This was confirmed statistically with no effect of Drug ($F<1$), no effect of Trial [$F(2,63)=2.1$, $P=.14$] and no interaction ($F=1$). Surprisingly, freezing was already very high during the CS of Trial 1 prior to any shock delivery, suggesting that the high-pitch tone is an aversive stimulus per se. Compared with this level of freezing, no learning occurred although performance was not at asymptotic levels. Overall, acute nefiracetam treatment did not affect fear conditioning to the tone.

Freezing may be regarded as an active process of inhibiting all other activities, and therefore to be dependent on the general level of *activity*. Substantially more freezing may be recorded in hypoactive animals and this could confound learning-related changes. Activity was thus recorded in the first 2 min of Training Session 1 prior to any CS or US in the conditioning box and sampled as movements crossing a vertical line from right to left (left to right) and head movements crossing a horizontal line (rearings). Results summarised in Fig. 1C suggest no differences between groups and more vertical crossings. A 4×2 factorial ANOVA with Drug (four levels) and Movement direction (two levels) confirmed this impression. There was no main effect of Drug [$F(3,42)=2$, $P=.16$], an effect of Movement direction [$F(1,42)=6$, $P=.02$], but no interaction [$F(3,42)=1.5$, $P=.23$].

3.2. Experiment 1B: effect of chronic nefiracetam treatment and fear conditioning

Since the effects of acute nefiracetam administration were rather small, we reasoned that in line with previous work, a long-term chronic exposure to the nootropic might result in a

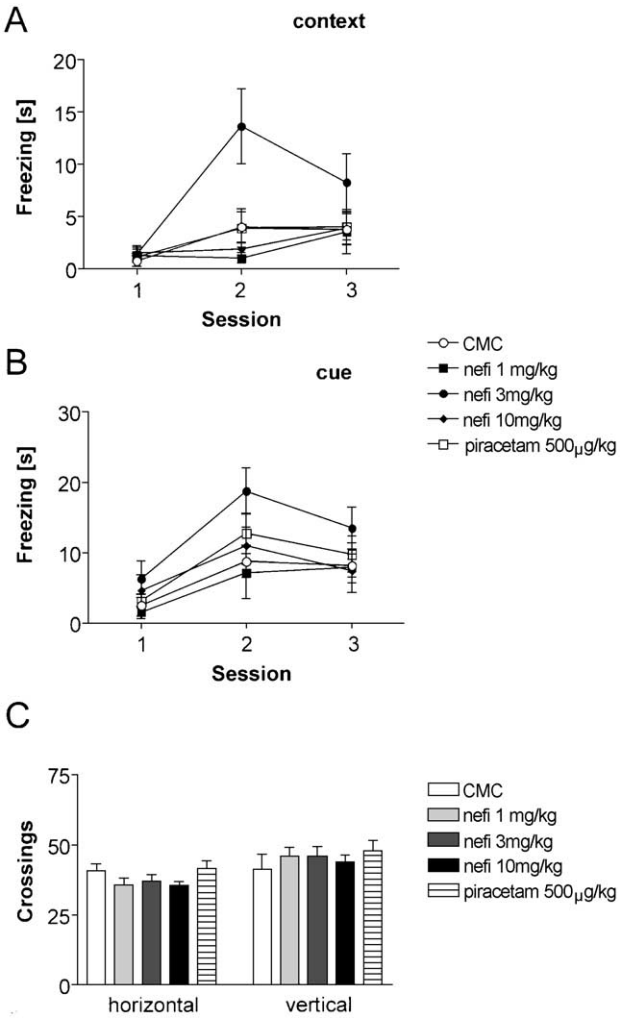

Fig. 2. Chronic effects of nefiracetam and piracetam on fear conditioning. Mean ± S.E.M. (A) Freezing to the context period (30 s) recorded during the first trial of each daily session. Vehicle (CMC), three different concentrations of nefiracetam and piracetam were orally applied for 16 days before conditioning. (B) Freezing to the cue (80 dB tone). (C) Activity measured as horizontal and vertical crossing during 300 s of habituation. Overall, nefiracetam in a dose of 3 mg/kg facilitated context conditioning. Piracetam and other concentrations of nefiracetam were ineffective.

more pronounced learning enhancement. Nefiracetam administration started 16 days prior to training and data are summarised in Fig. 2. For comparison, we added a group exposed to the well-known nootropic, piracetam, and administered 500 mg/kg, which was effective in our previous studies (Christoffersen et al., 1998). It is also noteworthy that we introduced a session for unconditioned suppression on Day 2 as a result of the high freezing levels obtained during the CS of Trial 1 in the previous experiment.

Freezing during the *context* progressively increased in all groups (Fig. 2A) but was enhanced in the 3-mg/kg nefiracetam group. All other groups were similar to the CMC-treated controls. This was confirmed statistically in a 5×3 factorial design yielding a main effect of Drug [$F(4,84) = 2.9$, $P = .04$], of Trial [$F(2,84) = 11.7$, $P < .0001$] and an interaction [$F(8,84) = 3.55$, $P = .001$]. These data suggest that while there is an overall increase in freezing, the groups also differ with respect to specific trials. Planned comparisons between the individual groups revealed a significant difference between the 3-mg/kg nefiracetam group and all other drug groups (all Fs > 9, Ps $< .005$), and all other groups did not differ from controls (all Fs < 1). Bonferroni posttests finally showed that freezing in the 3-mg/kg nefiracetam group did not differ from the other groups on Trial 1 prior to conditioning and Trial 5, but was significantly enhanced during Trial 3 ($P < .01$).

For the *cue* period, we obtained a similar pattern with the 3-mg/kg nefiracetam group outperforming all other subjects (Fig. 2B). While there was a main effect of Trial [$F(2,84) = 30.7$, $P < .0001$], no other factor reached significance (Fs < 1.2), suggesting that the drug groups did not differ significantly. A two-way ANOVA limited to the CMC controls and the 3-mg/kg nefiracetam group, however, revealed a significant effect of Drug [$F(1,21) = 8.6$, $P = .008$]. No other group differed from controls (all Fs < 1.5).

Activity was monitored during the initial 5 min of habituation to the conditioning chamber, i.e., after 2 weeks of drug exposure. There were considerably more vertical crossings [$F(1,56) = 27.8$, $P < .0001$], but this was similar in all groups ($F < 1$) and there was no interaction [$F(4,56) = 1.8$, $P = .14$]. In summary, chronic exposure to both nootropics did not cause hypoactivity.

3.3. Experiment 2: effect of chronic nefiracetam on AIDA-induced fear conditioning deficits

Given that chronic exposure to 3 mg/kg nefiracetam generated the most robust learning and memory enhancement of contextual fear conditioning, we chose this concentration and tested the hypothesis as to whether amnesia induced by mGluR Group I blockade, as seen in previous studies, could be attenuated or fully reversed. Two groups of animals were exposed to CMC or 3 mg/kg nefiracetam starting 7 days before habituation. During 4 days of training, we injected some animals intraperitoneally with saline or AIDA. Compared with Experiment 1, we also increased the shock intensity in order to replicate our older work (Nielsen et al., 1997; Christoffersen et al., 1999b). Results are summarised in Fig. 3.

For the *context* (Fig. 3A), freezing in the chronic 3-mg/kg nefiracetam/saline group was not enhanced compared with CMC controls. CMC/AIDA strongly impaired context conditioning while nefiracetam/AIDA enhanced freezing to context. A 4×4 factorial ANOVA with Drug (four levels) as between-subject and Trial (four levels) as within-subject factor revealed both a main effect of Drug [$F(3,152) = 9.8$, $P < .0001$] and of Trial [$F(3,152) = 12.5$, $P < .0001$], but failed significance for the interaction [$F(9,152) = 1.7$, $P = .09$]. Compared with the CMC/saline group, the CMC/AIDA group was significantly worse [$F(2,52) = 5.9$, $P = .02$], the nefiracetam/AIDA group was superior [$F(1,68) = 6.1$, $P = .02$] and the nefiracetam/saline group did not differ ($F < 1$).

With respect to freezing to the *tone* (Fig. 3B), all groups performed similarly with high levels of freezing already on Trial 3. Statistical analysis yielded a main effect of Trial [$F(3,152) = 42.9$, $P < .0001$], but no effect of Drug [$F(3,152) = 1.8$, $P = .14$] and no interaction [$F(9,152) = 1.2$, $P = .28$].

3.4. Drug treatment effects on Trial 2 performances

For completion, we also examined freezing in Trial 2, especially for the chronic nefiracetam (Experiment 1B) groups and the AIDA groups (Experiment 2). Statistical

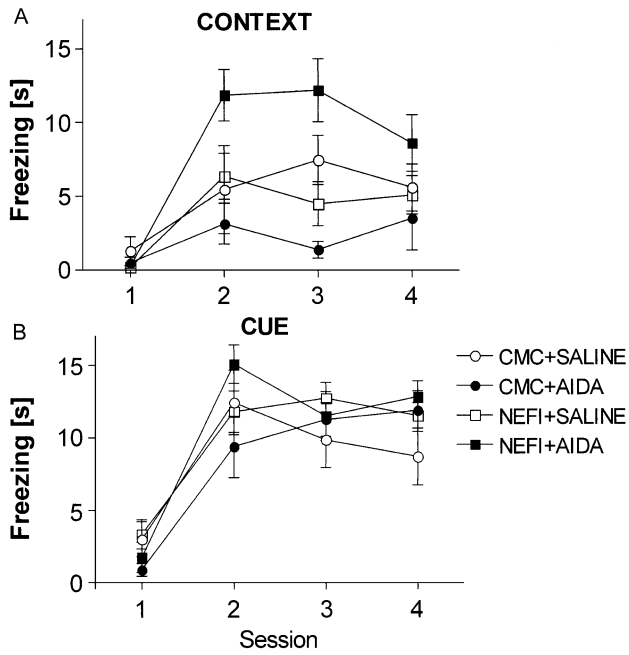

Fig. 3. Reversal of AIDA-induced contextual fear conditioning deficit by nefiracetam. Mean ± S.E.M. (A) Freezing to context was impaired in CMC + AIDA group compared with CMC + saline and nefiracetam + saline. Freezing in the nefiracetam – AIDA group was supranormal. (B) Freezing to the tone was not affected by any drug treatment.

analyses revealed no main effects of Drug in any experiment ($P > .05$; data not shown). This was most likely due to the high amounts of freezing in controls.

4. Discussion

4.1. mGluRs mediate memory formation and neurotoxicity

mGluRs have been repeatedly shown to be involved in memory formation. Initial experiments have used broad-spectrum antagonists like MCPG (Riedel et al., 1994, 1995; Richter-Levin et al., 1994). Since then, more potent and selective antagonists for all subtypes have emerged, and work using AIDA, 4-CPG and 2-methyl-6-(phenylethynyl)-pyridine (MPEP) as selective Group I mGluR antagonists has consistently resulted in memory impairments (Nielsen et al., 1997; Christoffersen et al., 1999b; Balschun and Wetzel, 1998; Balschun et al., 1999; Schulz et al., 2001). In line with these reports, we confirmed our previous work of a selective contextual, but not cue conditioning, deficit in AIDA-treated rats. Interestingly, there was no deficit in Trial 2 on each day (data not shown), suggesting normal within-session learning/short-term memory (Christoffersen et al., 1999a,b). Nevertheless, amnesia was monitored on Trial 1 each day, which supports the notion that there was no acquisition, but rather a consolidation or retrieval deficit. At present, however, we cannot distinguish between the two possibilities. Work that has used different infusion protocols, however, strongly suggests that mGluRs are involved in both memory consolidation and retrieval (Barros et al., 2000; Izquierdo et al., 2000).

The selective impairment in freezing to context is consistent with blockade of hippocampal mGluRs rather than blockade of extrahippocampal mGluRs, as has been suggested by lesion studies reporting a context-specific freezing deficit after hippocampal removal but no effect on cue conditioning (Kim and Fanselow, 1992; Phillips and LeDoux, 1992, 1995). While the hippocampus in rats may be important for multiple stages of memory formation (Riedel et al., 1999), it would appear that Group I mGluRs mediate long-term consolidation (Riedel et al., 2000). Although we have not performed direct intrahippocampal infusions to verify this hypothesis, Ohno and Watanabe (1996, 1998) have administered AIDA at a dose of 3.2 μg/μl into each hippocampus and found that spatial working memory was severely compromised.

Apart from this function in normal synaptic transmission and synaptic plasticity, overstimulation of mGluRs can induce neurotoxicity. The selective blockade of Group I mGluRs, in particular mGluR5, has been proposed as a means to prevent such neurotoxicity from occurring (Bruno et al., 1999, 2000; Schröder et al., 1999; Strasser et al., 1998). If therapeutically successful, this intervention would be at the expense of normal memory formation and such a side effect should be avoided: combining therapy with memory-enhancing agents may, therefore, be the preferred solution.

4.2. Nefiracetam recovers memory in AIDA-exposed amnesic rats

Nootropics are pharmacologically active compounds occupying a special position in pharmacology of the central nervous system. Positive effects have been reported in a number of experimental situations, which may be due to one or multiple chemical actions (for review, see Gouliaev and Senning, 1994). Despite some variability in their actions, nootropics are virtually free of toxic effects and, in the case of nefiracetam, exert little, if any, effect on nonneuronal tissue (Kitano et al., 1994). Preclinical and clinical studies on nefiracetam have advanced recently and nefiracetam has been in Phase II clinical trials since 1994 in patients with Alzheimer's dementia. Its mode of action has many facets and it interacts with cholinergic (Hiramatsu et al., 1992; Kawajiri et al., 1994; Zhao et al., 2001), monoaminergic (Luthman et al., 1991) and GABAergic transmission (Watabe et al., 1993). In addition, nefiracetam increases long-lasting N/L-type calcium channel currents (Yoshii et al., 1997; Hiramatsu et al., 1997), suggesting enhanced release of neurotransmitters. In contrast to other racetams, namely piracetam, oxiracetam and aniracetam, understanding of interactions of nefiracetam with glutamatergic transmission remains elusive (Gouliaev and Senning, 1994).

Interestingly, we report on one of the rare cases (Experiment 1B) in which nefiracetam facilitated learning/memory formation in normal young animals (Sakurai et al., 1989). In most cases, nefiracetam shows its potential by attenuating the amnesic effects induced by drugs like scopolamine (Sakurai et al., 1989) or various morphines (Nabeshima, 1994), by ageing (Hasegawa et al., 1996; Woodruff-Pak and Li, 1994), cerebral embolism (Tanaka et al., 1992) or brain lesions (Hiramatsu et al., 1997). It may, thus, not be surprising that nefiracetam also reverses the AIDA-induced memory deficit. An as yet unexplained result, however, is the magnitude of this reversal. AIDA injected into chronically with nefiracetam-treated animals caused memory facilitation, even when compared with CMC-treated controls or nefiracetam alone (Fig. 3). One possible, but rather speculative, interpretation would be that when applied to the intact brain, nefiracetam might activate multiple transmitter systems (see above). Hyperactivation, however, may be counteracted by the simultaneous increase in glutamatergic and thereby mGluR activity, which, when blocked by means of AIDA, may enable enhancement of cholinergic and/or monoaminergic transmission. Such enhanced nonglutamatergic transmission would increase attention and eventually result in better task performance due to memory enhancement.

The exact location where nefiracetam may act in the brain is difficult to determine after systemic application of the drug. Interest here is focused on the hippocampus given that AIDA selectively blocked freezing to the context and not to

the tone. This could be interpreted as a hippocampal action, and it would be of interest to evaluate the actions of nefiracetam on hippocampal responses. Recent evidence suggests that it can act on the hippocampal circuitry in order to alleviate ageing symptoms in rabbits (Woodruff-Pak, 1997; Woodruff-Pak et al., 1997). Moreover, physiological data obtained from hippocampal slices have provided evidence for a nefiracetam-induced long-lasting potentiation in CA1 pyramidal neurones, which occludes with tetanus-induced long-term potentiation, but is independent of *N*-methyl-D-aspartate (NMDA) receptor activation (Nishizaki et al., 1999) and requires activation of nicotinic acetylcholine receptors (Nishizaki et al., 1998, 2000; Yoshii et al., 2001). Also, there is no effect on α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Nomura and Nishizaki, 2000), suggesting actions different from piracetam (Cohen and Müller, 1993) and aniracetam (Tsuzuki et al., 1992; Xiao et al., 1991). Effects on mGluRs remain elusive at this stage. It seems clear, however, that while blockade of mGluRs in general impaired synaptic plasticity (Riedel et al., 1995), AIDA and other Group I antagonists had mixed effects (McCaffery et al., 1998; Balschun et al., 1999), but nefiracetam strongly enhanced neuronal activity (Nishizaki et al., 1999). How this combination would lead to supranormal memory warrants further studies.

5. Conclusions

We present evidence for a selective contextual memory impairment in AIDA-treated rats, which suggests an important role of hippocampal Group I mGluRs in this task. Blockade of Group I mGluRs, however, is a potential clinical treatment for prevention of neurotoxicity, but amnesia as a side effect of such therapy should be avoided. One possible strategy is the use of memory-enhancing agents — nootropics. Nefiracetam, a potent nootropic agent, fully reversed the AIDA-induced deficit into a supranormally high performance level. The underlying physiological and pharmacological mechanisms of this effect remain to be investigated.

Acknowledgments

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