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Short communication

Acute ampakine treatment ameliorates age-related deficits in long-term potentiation

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ABSTRACT

Memory loss observed as a consequence of aging is paralleled by a down-regulation of AMPA-type glutamate receptors (AMPARs) that mediate fast excitatory synaptic transmission. Activation of these receptors enhances long-term potentiation (LTP), a neuronal process demonstrated to be crucial for memory storage and thought to be a cellular substrate of learning and memory. In the present studies, we determined that LTP was reduced in aged rats when compared to young rats and that acute treatment with CX1846, a novel AMPAR positive allosteric modulator, fifteen minutes prior to tetanic stimulation completely reversed the significant deficit in LTP observed in aged rats. These results suggest that CX1846 might be useful for the treatment of age-related memory impairments.

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1. Introduction

Long-term potentiation (LTP) has been vigorously studied due to its apparent involvement in synaptic plasticity [1] and has been proposed to be a cellular substrate of learning and memory [2]. LTP is defined as an enduring increase in the amplitude of excitatory postsynaptic potentials following high-frequency (tetanic) stimulation of afferent pathways. Ionotropic AMPA glutamate receptors (AMPARs) that mediate fast EPSPs have been shown to be crucial for mediating LTP in multiple brain regions [3], including the hippocampus [4] and the dorsal striatum [5], to a considerably greater extent than NMDA glutamate receptors [4,5].

AMPAR activation has been linked to synaptic tuning and swift modifications to dendritic spine actin networks, a cellular process necessary for the consolidation of LTP [6]. In addition, LTP is accompanied by the release of brain-derived neurotrophic factor (BDNF), a protein responsible for neuronal survival, development and stabilization of the aforementioned dendritic spine modifications [7].

Several age-related alterations involving LTP have been reported [2,8,9] that may have important relationships to the age-related cognitive decline observed across species. In parallel with the age-related decline in LTP, diminished surface expression of glutamate receptors has been reported in several brain regions in aged rodents [3,8,10–13] and humans [14]. Conversely, stabilizing GluA1 surface expression in the hippocampus of aged rats has been reported to attenuate age-related impairments of LTP [2].

Ampakines are positive allosteric modulators of the AMPAR and exert their effects by holding the receptor in the ligand-bound, open channel conformation, increasing agonist binding affinity and thereby offset desensitization of the receptor. This drug class has been shown to have disease-modifying activities in animal models of stroke [15], Rett syndrome [16], Huntington's Disease [6] and autism [17]. Given the intimate relationship between AMPARs and LTP, the present study sought to determine the effects of CX1846, a potent, high impact ampakine (Type I according to the designation of Arai et al. [18]) on LTP in both young and old rats. CX1846 was chosen due to its high bioavailability (~99%) and long half life (~1.6 h).

2. Materials and methods

All animal procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and with protocols approved by the Institutional Animal Care and Use Committee of the University of California at Irvine (Irvine, CA). Efforts were made to minimize animal suffering and the numbers of rats used in the work described. CX1846 ((*R*)-8-(1-(2*H*-tetrazol-2-yl)propan-2-yl)-3-cyclopropyl-3,8-dihydro-[1,2,3]triazino[4,5-*g*]quinazoline-4,9-dione) was synthesized at

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RespireRx Pharmaceuticals Inc. and prepared for injection by solubilizing in saline containing 33% HPCD (Hydroxypropyl-P-cyclodextrin).

Fisher F344/N rats (250–350 g) were anesthetized by a 60 mg/kg intraperitoneal injection of pentobarbital. Young rats were defined as 2 months of age, while old rats were defined as 20–21 months of age. For drug administration and blood sampling, two catheters made of polyethylene tubing (PE10) were inserted into the femoral vein and artery, respectively. Animals were maintained under anesthesia by pentobarbital infusion at a rate of 2–4 mg/kg/h. After the animal was placed into a stereotaxic frame, small holes were drilled into the skull of the left hemisphere to allow the positioning of a stimulating electrode (−7.8 to −8.1 from bregma, 4.2 to 4.4 lateral to midline) and a recording electrode (−3.0 to −3.3 from bregma, 1.6 to 2.2 lateral to midline). A monopolar stainless steel stimulating electrode (175 m, insulated with formvar) was lowered into the perforant path together with a platinum/iridium recording electrode (75 m) into the hilus of the dentate gyrus of the hippocampus. Evoked excitatory post-synaptic field potentials (EPSPs) were recorded in response to single pulse stimulation delivered to the perforant path at a frequency of one pulse per 20 s and peak amplitudes were determined using commercially available data acquisition and analysis software (NAC and NACSHOW). The current used to elicit the EPSPs was adjusted to produce a response size of 50–60% of the maximal spike-free amplitude.

After a stable baseline had been established, baseline EPSPs were recorded for 10 min and then CX1846 or vehicle was injected intraperitoneally and EPSPs were recorded for a further 85 min. Fifteen minutes after the injection, a tetanic protocol (20 trains at 400 Hz of 30 ms duration with current intensity increased to 80% of the maximal response) was used to produce LTP. Field potential recordings were continued at the same rate and intensity of stimulation as during the baseline period.

LTP was quantified by determining the means ± standard errors of the peak amplitudes of the EPSPs (expressed as a per cent of baseline) produced 70 min after the tetanizing stimulation. The effects of age and CX1846 on LTP was evaluated by one-way analysis of variance and unpaired student's *t*-test [20].

3. Results

In young rats that received the vehicle control, tetanizing stimulation produced the typical reported pattern of LTP with an almost immediate potentiation of the EPSPs, a decline over the first 5 min and then a slower decline over the next 60 min (see Fig. 1A). One hour after tetanizing stimulation, the mean peak EPSP amplitude was still approximately 14% greater than baseline ($p < 0.01$, *t*-test, n = 9). Administration of CX1846 produced a dose-related increase in the mean peak EPSP amplitude during the course of the entire recording session so that by one hour after tetanizing stimulation, the mean peak EPSP amplitudes for 0.3 and 1 mg/kg of CX1846 were approximately 115% ($p < 0.01$, *t*-test, n = 6) and 240% ($p < 0.001$, *t*-test, n = 6) greater than that produced by vehicle, respectively (see Figs. 1A and 2). CX1846 did not significantly enhance EPSP during before tetanus ($p = 0.81$, *t*-test, n = 6).

Compared to young rats, tetanizing stimulation in vehicle-treated old rats produced significantly less potentiation, particularly during the second half of the recording session (see Fig. 1B). When measured one hour after tetanizing stimulation, the mean peak EPSP amplitude recorded from vehicle-treated old rats was approximately 6% greater than baseline ($p < 0.05$, *t*-test, n = 6), but significantly ($p < 0.01$, *t*-test, n = 6) smaller than that observed in vehicle-treated young rats (approximately 14% greater than baseline).

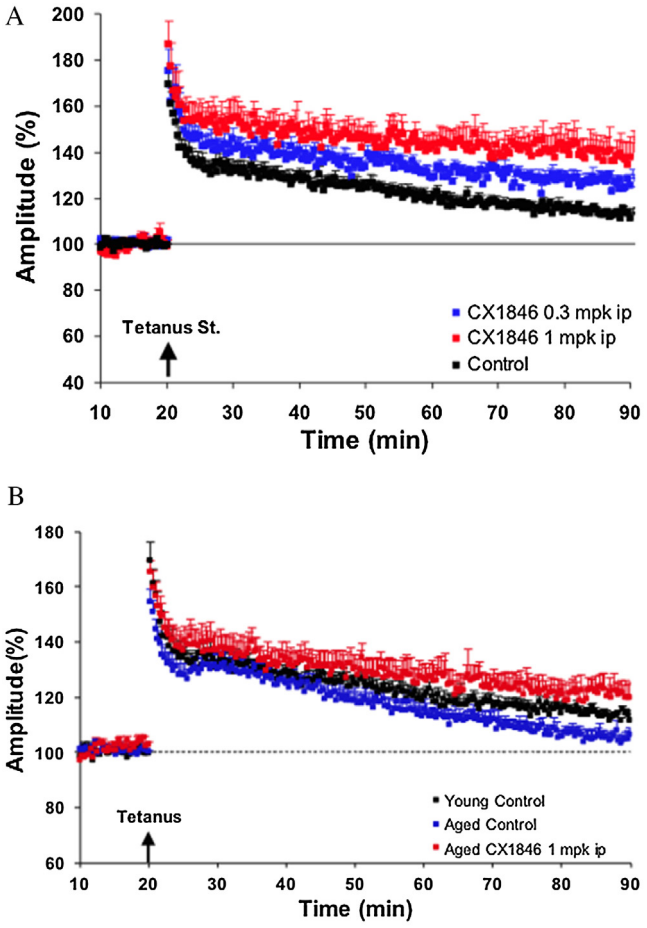

Fig. 1. A) Dose-dependent effects of CX1846 on LTP in young rats. The time course of the measured increase in the amplitude of the EPSP in dentate gyrus following stimulation of the perforant path in the rat hippocampus. Data points represent the mean and standard error from 6 to 9 animals. B) Effects of CX1846 on LTP in old rats. The time course of the measured increase in the amplitude of the EPSP in dentate gyrus following stimulation of the perforant path in the rat hippocampus. Data points represent the mean and standard error from 6 to 9 animals.

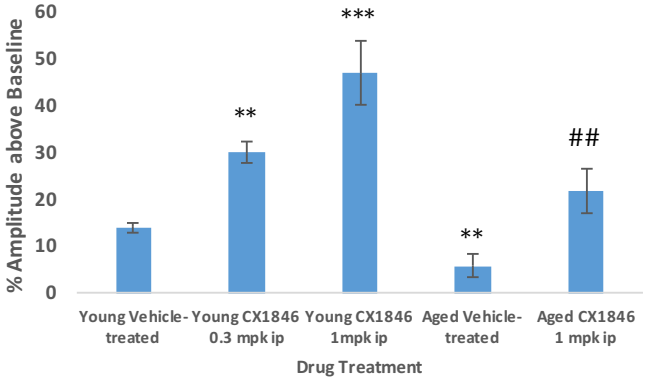

Fig. 2. The mean percent increase in amplitude of the LTP for each treatment measured 60–70 min after tetanic stimulation. Data points represent the mean and standard error from 6 to 9 animals. ** $P < 0.01$, *** $P < 0.001$, following analysis using a one-way ANOVA and *t*-test versus control. ## $P < 0.01$, following analysis using a one-way ANOVA and *t*-test versus aged-rat control.

Administration of 1 mg/kg of CX1846 to old rats, increased the mean peak EPSP amplitude during the course of the entire recording session (see Fig. 1B), so that by one hour after tetanizing stimulation, the mean peak EPSP amplitude recorded after CX1846

was significantly greater than that produced by vehicle (21% vs 6%, $p < 0.01$, *t*-test) (see Fig. 2). These normalized mean peak EPSP amplitudes produced by CX1846 were comparable to those observed in the vehicle treated young rats (14% and 22% for young and old rats, respectively; $p = 0.1$, *t*-test). While the administration of 1 mg/kg of CX1846 to old rats produced a subtly greater mean peak EPSP amplitudes compared to those recorded from vehicle-treated young rats, it still produced significantly smaller mean peak EPSP amplitudes compared to comparably treated young rats (22% vs 47%, $p < 0.01$, *t*-test, $n = 6$). However, because of the differences in the mean peak EPSP amplitudes recorded from the vehicle-treated rats in the two age groups, the percent enhancement produced by CX1846 compared to vehicle was comparable in the two age groups, 240% and 260% for young and old rats, respectively ($p < 0.3$, *t*-test, $n = 6$). It is also of considerable interest that in both young and old rats, administration of CX1846 did not produce the robust epileptiform observed by its predecessor compound Cyclothiazide [19] a characteristic of Class I ampakines that has previously limited their clinical feasibility. This finding also demonstrates that the therapeutic effect of ampakines can be separated from their undesired convulsant effects.

4. Discussion

The etiogical role of LTP in regards to memory formation has yet to be fully elucidated. There is a growing body of evidence to suggest that memory dysfunction and LTP decline are associated with aging [2,8]. Numerous reports have also noted the loss of AMPA-type glutamate receptors in multiple brain regions during aging [8,12,13]. It previously has been established that positive allosteric modulation of AMPARs by ampakines augmented LTP of 8–10 month old rats in parallel with an increase in BDNF expression [20], though, it should be noted that Rex et al. did not demonstrate these middle-aged rats experienced significant reductions in LTP compared to their younger age counterparts [20].

In confirmation of prior work reporting the ability of certain ampakines to enhance LTP in young rats [21,22], the present studies demonstrate a dose-related enhancement of LTP produced by CX1846, a high impact ampakine (Type I according to the designation of Arai et al. [18]), when compared to vehicle-treated young rats. Extending previous reports of LTP deficits in middle-aged rats 22 months of age [2], the present studies demonstrate significantly less LTP in 20–21 month old, vehicle-treated old rats, when compared to young rats. In addition, administration of CX1846 to these old rats significantly enhanced LTP when compared to vehicle-treated old rats. The LTP increase was comparable to that observed in vehicle-treated young rats, but less than young rats treated with CX1846. Since the present studies only used one dosage strength, it is uncertain whether higher doses given to old rats might have increased LTP even more.

While certain ampakines also have been reported to stimulate production of BDNF [15,23–25], the role of BDNF in mediating ampakine enhancement of LTP is unclear. In a slice preparation, ampakine-stimulated increases in BDNF mRNA have been reported 3 h after drug administration with maximal increases in neurotrophin mRNA occur after 24–48 h [25]. In the present study, drug-induced enhancement of LTP can be observed in both young and old rats as early as 30 min after administration of CX1846, presumably prior to the major rise in BDNF.

It is still of considerable interest that hippocampal LTP can be modulated in aged mice without increased BDNF expression. All that seems to be required to correct the age-related deficit in LTP in the hippocampus is augmentation of AMPAR-mediated currents, a conclusion supported by Lin et al. [4], who found that AMPAR activation is necessary to observe LTP in the hippocampus. A recent investigation into the mechanism of LTP consolidation concluded that afferent stimulation induces the release of BDNF, which stabilizes cytoskeletal changes associated with LTP induction [7]. Therefore, it may be possible that amplifying AMPAR-mediated currents causes a greater release of BDNF, resulting in greater LTP induction, while not necessarily modulating neurotrophin gene expression. We do not believe based upon screens with predecessor compounds that CX1846 enhances the activity of alternate neurotransmitter systems (data not shown), though future studies will be done to delineate the possibility.

Although CX1846 only seems to enhance AMPAR-mediated currents in the results described here, this novel and potent ampakine may have multiple disease modifying effects by facilitating AMPAR-mediated currents and enhancing neurotrophin expression. Induction of BDNF seems to be crucial in correcting neurological deficits and losses of synaptic plasticity in multiple disease or injury models such as Alzheimer's Disease [26,27], Huntington's Disease [6], Rett Syndrome [16], Fragile X Syndrome [28] and stroke [15] in addition to being able to protect against MPP+ toxicity [23]. This may be of substantial clinical interest, given that if 1 mg/kg CX1846 more than corrects the impairment in LTP in aged rats, it may also be sufficient to safely augment BDNF levels in the aforementioned neurological disease models. Studies are currently underway to examine the behavioral effects of CX1846 administration in young and aged rats. Preliminary studies suggest that at doses comparable to those that enhance LTP in young and aged rats, CX1846 may enhance performance of rats in the 8-arm radial maze, a method for assessing working memory, which often deteriorates in Alzheimer's patients [13], further supporting the use for ampakines in the treatment of age-related memory impairment.

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