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The Trace Kynurenine, Cinnabarinic Acid, Displays Potent Antipsychotic-Like Activity in Mice and Its Levels Are Reduced in the Prefrontal Cortex of Individuals Affected by Schizophrenia

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Cinnabarinic acid (CA) is a kynurenine metabolite that activates mGlu4 metabotropic glutamate receptors. Using a highly sensitive ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS-MS) method, we found that CA is present in trace amounts in human brain tissue. CA levels were largely reduced in the prefrontal cortex (PFC) of individuals affected by schizophrenia. This reduction did not correlate with age, sex, duration of the disease, and duration and type of antipsychotic medication and might, therefore, represent a trait of schizophrenia. Interestingly, systemic treatment with low doses of CA (<1 mg/kg, i.p.) showed robust efficacy in several behavioral tests useful to study antipsychotic-like activity in mice and rats and attenuated MK-801-evoked glutamate release. CA failed to display antipsychotic-like activity and inhibit excitatory synaptic transmission in mice lacking mGlu4 receptors. These findings suggest that CA is a potent endogenous antipsychotic-like molecule and reduced CA levels in the PFC might contribute to the pathophysiology of schizophrenia.

Key words: kynurenine pathway/metabotropic glutamate receptor/mood disorder/human tissue/endogenous metabolite/behavior/HPLC-mass/mass/electrophysiology/MK-801

Introduction

There is increasing interest on the kynurenine pathway (KP) of tryptophan metabolism since the early times of its characterization.[1–3] The KP is linked to the pathophysiology of schizophrenia and might be targeted by therapeutic intervention.[4–7] Three enzymes, type-1 and -2 indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase, convert L-tryptophan into formylkynurenine, which is transformed into L-kynurenine. L-Kynurenine is converted into 3-hydroxykynurenine by kynurenine monooxygenase (KMO), or, alternatively, transaminated into kynurenic acid (KYNA) by kynurenine aminotransferase. 3-Hydroxyanthranilic acid (3-HANA) and quinolinic acid (QUINA) are sequentially synthesized from 3-hydroxykynurenine by kynureninase and 3-hydroxyanthranilic acid dioxygenase, respectively.[8,9] KYNA is an antagonist at the glycine site of N-methyl-D-aspartate (NMDA) receptors, whereas QUINA activates NMDA receptors.[10–12] Proinflammatory cytokines, elevated in schizophrenia, activate the KP.[5,8]

KMO activity is defective in the brain of individuals with schizophrenia,[7] and polymorphic variants of its gene are associated with schizophrenia.[13] In addition, mice lacking KMO develop a behavioral phenotype recapitulating the hallmark features of schizophrenia.[14] A reduced activity of KMO leads to an increased KYNA:QUINA ratio, thus supporting the hypothesis of NMDA receptor hypofunction in schizophrenia.[15–18] Accordingly, both cerebrospinal fluid and brain tissue KYNA levels are consistently increased in individuals with schizophrenia.[19,20]

The connection between the KP and schizophrenia may involve molecules other than KYNA and QUINA. Xanthurenic acid (XA) and cinnabarinic acid (CA) are generated by transamination of 3-hydroxykynurenine and condensation of 2 molecules of 3-HANA, respectively. XA levels are reduced in the blood of schizophrenic patients and their relatives, and administration of XA attenuates MK-801-induced hyperactivity in mice, a behavioral test predictive of antipsychotic-like activity.[21] CA is an orthosteric agonist of mGlu4 metabotropic glutamate receptors,[22] binds the arylic hydrocarbon receptor (AhR),[23] and inhibits indoleamine 2,3-dioxygenase.[24]

Pharmacological activation of mGlu4 receptors produces antipsychotic-like effects in rodents.[25–27] Furthermore, CA restrains neuroinflammation,[28] which is consistently observed in brains of schizophrenic patients at times preceding the first-episode of psychosis.[29–33] This gave us the impetus to study CA in animal models predictive of antipsychotic-like activity and to measure endogenous CA levels in the prefrontal cortex (PFC) from individuals with schizophrenia and age-matched controls.

Materials and Methods

Materials

See supplementary material.

UPLC/MS-MS Analysis of CA in the Human PFC

We measured CA levels in PFC samples from individuals with schizophrenia and non-schizophrenic controls obtained from the Harvard Brain Tissue Resource Center, funded through NIH-NeuroBiobank HHSN-271-2013-00030C (see supplementary material). Briefly, after brain tissue was homogenized, supernatant was used for UPLC analysis with a reversed-phase column (100 × 2.1 mm, Luna Omega 1.6 µm PS-C18, 100 Å; Phenomenex). Mass spectrometry was performed on hybrid triple quadrupole/linear ion trap mass spectrometer (QTRAP 5500; SCIEX), equipped with Turbo Ion Spray source. The detector was set in positive ion mode. Ion spray voltage was set at 5500 V and the source temperature was 450°C. The instrument was set in the multiple reaction monitoring mode, monitoring the transitions *m/z* 301.2-264.7, 301.2-237.4, and 301.2-209.4. Mass spectrometer parameters were optimized to maximize sensitivity for all transitions (supplementary table S2). We used the same method to measure the CA levels in the serum and brain tissue of mice treated with CA (see below); MS analysis of serum samples was performed using a 6470-quadrupole system (Agilent technologies).

Animal Studies

We used adult male C57BL6/J mice (22–24 g; Charles River) for measurements of CA, immunohistochemistry, microdialysis, neuronal K^+/Cl^- symporter (KCC2) immunoblotting, electrophysiology, studies on synaptosomes, and all behavioral experiments except the pre-pulse inhibition (PPI) test. Assessment of MK-801-induced hyperactivity and electrophysiological analysis were also performed in $mGlu4^{-/-}$ mice (on C57BL6/J background), originally purchased from The Jackson Laboratory (Bar Harbor). PPI was assessed in male Wistar rats (220–255 g; Charles River). All animals were housed (10 mice or 4 rats/cage) under standard conditions with a 12-h light/dark cycle. Behavioral experiments were performed after a period of acclimatation and handling. Drugs were administered i.p. in a volume of 10 ml/kg in mice and 1 ml/kg in rats.

In vivo studies were performed in accordance with the European Communities Council Directive of September 22, 2010 (2010/63/EU), Polish legislation acts concerning animal experimentation, and the Italian Guidelines for Animal Use and were approved by the Local Ethics Committee by the Institute of Pharmacology, Polish Academy of Sciences in Krakow, and the Local Ethics Committee of I.R.C.C.S. Neuromed. All efforts were made to minimize the number of animals.

Measurements of CA Levels in Mice

CA levels were measured in serum, cerebral cortex, and cerebellum of mice receiving a single i.p. injection of CA (0.25 mg/kg) and killed at different timepoints. Control mice were treated with saline and killed after 1 hour. CA levels were measured by ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS-MS), as detailed above.

Immunofluorescence in Mouse Brain Tissues

Mice were injected i.p. with either saline, lipopolysaccharide (LPS, 10 mg/kg), or CA (0.5 mg/kg) and killed at different timepoints. We used LPS to induce neuroinflammation and activate the KP. Brain sections were incubated with mouse monoclonal anti-CA antibodies (1:100, ImmuSmol) and secondary antibodies conjugated with Alexa-Fluor 488 (1:200, Invitrogen). Sections were examined with a ZEISS-780 confocal laser scanning microscope (see supplementary material).

Behavioral Tests Predictive of Antipsychotic Activity

All behavioral tests were performed as described previously[21,34] and detailed in the supplementary material. For measurements of MK-801-induced hyperactivity, mice were pretreated with different doses of CA (from 0.125 to 20 mg/kg) followed, 1 h later, by a single injection of MK-801 (0.32 mg/kg). Experiments were also performed with the AhR inhibitor, CH-223191 (1-Methyl-*N*-[2-methyl-4-[2-(2-methylphenyl)diazenyl]phenyl-1H-pyrazole-5-carboxamide), and in mGlu4$^{-/-}$ mice treated with CA. The PPI test was performed in rats treated with 3 doses of CA (0.125, 0.25, or 0.75 mg/kg) followed by either MK-801 (0.32 mg/kg) or saline. The test consisted of 2 trials in which a 120 dB/40 ms pulse was first delivered alone and then preceded by a prepulse of 75 dB/20 ms. The amplitude of the startle response was measured as detailed. The novel object recognition test was performed in mice. In the training trial, performed 1 day after the habituation trial, mice were allowed to explore 2 identical objects for 5 minutes. In the test trial, performed 1 hour later, one familiar object was replaced by a novel object, and 5 more minutes were allowed for exploration. Mice were treated with saline or 3 doses of CA (0.625, 0.125, or 0.25 mg/kg) 1 h prior to MK-801 (0.32 mg/kg) or saline. In the social interaction test, mice housed in separate cages were allowed to explore a black plastic box (50 × 30 × 35 cm) illuminated with a light intensity of 335 lux (habituation trial). In the trial-test, each mouse pair was placed in the box for 5 minutes and social interaction was recorded. CA (0.065, 0.125, 0.25, and 20 mg/kg) was administered 1 h prior to MK-801 (0.32 mg/kg, i.p.). In the head twitch test, mice were treated with CA (0.125, 0.5, or 5 mg/kg) followed, 1 h later, by (+/−)-2,5-dimethoxy-4-iodoamphetamine (DOI, 2.5 mg/kg, i.p.). Episodes of head twitches were counted for 20 minutes.

Microdialysis in Freely Moving Mice

Microdialysis in freely moving mice and measurements of glutamate levels were performed as described previously (see supplementary material).[35,36] Mice were injected with saline or CA (0.5 mg/kg, i.p.) and fractions were collected for 60 minutes. Mice then received MK-801 (0.32 mg/kg, i.p.) and fractions were collected for an additional 140 minutes.

Western Blot Analysis of KCC2 Levels

Immunoblot analysis of KCC2 in mouse PFC was performed as described previously (see supplementary material).[37] Mice were pretreated i.p. with CA (0.5 mg/kg) or saline followed, 1 h later, by either saline (group pretreated with the vehicle) or MK-801 (0.32 mg/kg).

In Vitro Studies

Measurement of cGMP Formation in Cultured Cerebellar Granule Cells. Primary cultures of rat cerebellar granule cells were prepared from 8-day-old rats, and cyclic guanosine monophosphate (cGMP) were measured as described previously (see supplementary material).[38]

Electrophysiological Analysis of Synaptic Transmission. Excitatory postsynaptic currents (EPSCs) were recorded from layer 5 pyramidal neurons (L5) in PFC slices from 2-month-old wild-type and mGlu4$^{-/-}$ mice. Cells were recognized by their characteristic pyramidal soma and spike shape, and only cells with resting membrane potential ≤−60 mV before drug application were used for the experiments. EPSPs were recorded at 25°C using a Multiclamp 700-A amplifier (Axon Instruments), at −70 mV holding potential using a KCl-based internal solution in the presence of bicuculline (20 µM). CA (1 µM) was applied by bath perfusion for 5 minutes (see supplementary material).

Preparation of Synaptosomes and Release Experiments. Preparation of cortical synaptosomes and measurements of [^{3}H]D-aspartate and [^{3}H]GABA release in response to depolarizing concentrations of K^+ and different concentrations of CA were performed as described previously (see supplementary material).[39]

Statistical Analysis

One-way ANOVA followed by Dunnett's test was used for the analysis of behavior, KCC2 protein, electrophysiology, and experiments in synaptosomes. Microdialysis data were analyzed by 1-way ANOVA for repeated measures followed by Fisher's tests. CA data in human cortical samples were analyzed by Student's *t*-test. Correlation between CA levels and different variables was studied by linear regression analysis.

Results

Reduced Endogenous Levels of CA in the PFC of Individuals Affected by Schizophrenia

We developed a highly sensitive UPLC/MS-MS method detecting picogram (pg) of CA in brain tissue. CA levels were measured in PFC samples from 23 individuals with schizophrenia (16 males and 7 females; age, 25–61 y) and 26 non-schizophrenic controls (22 males and 4 females; age, 36–63 y). In 15 individuals with schizophrenia, disease duration ranged from 7 to 46 years; one presented major depression for 15 years and schizophrenia for 1 year. Disease duration was not reported in all other individuals. Information on the treatment was available in 16 individuals with a polytherapy history of classical or atypical antipsychotics sometimes combined with valproate, lithium, or doxepine. Treatment duration ranged from 1 to 30 years. Postmortem intervals ranged from 6.1 to 19.9 hours. Neuropathological examination showed signs of cerebrovascular disorders

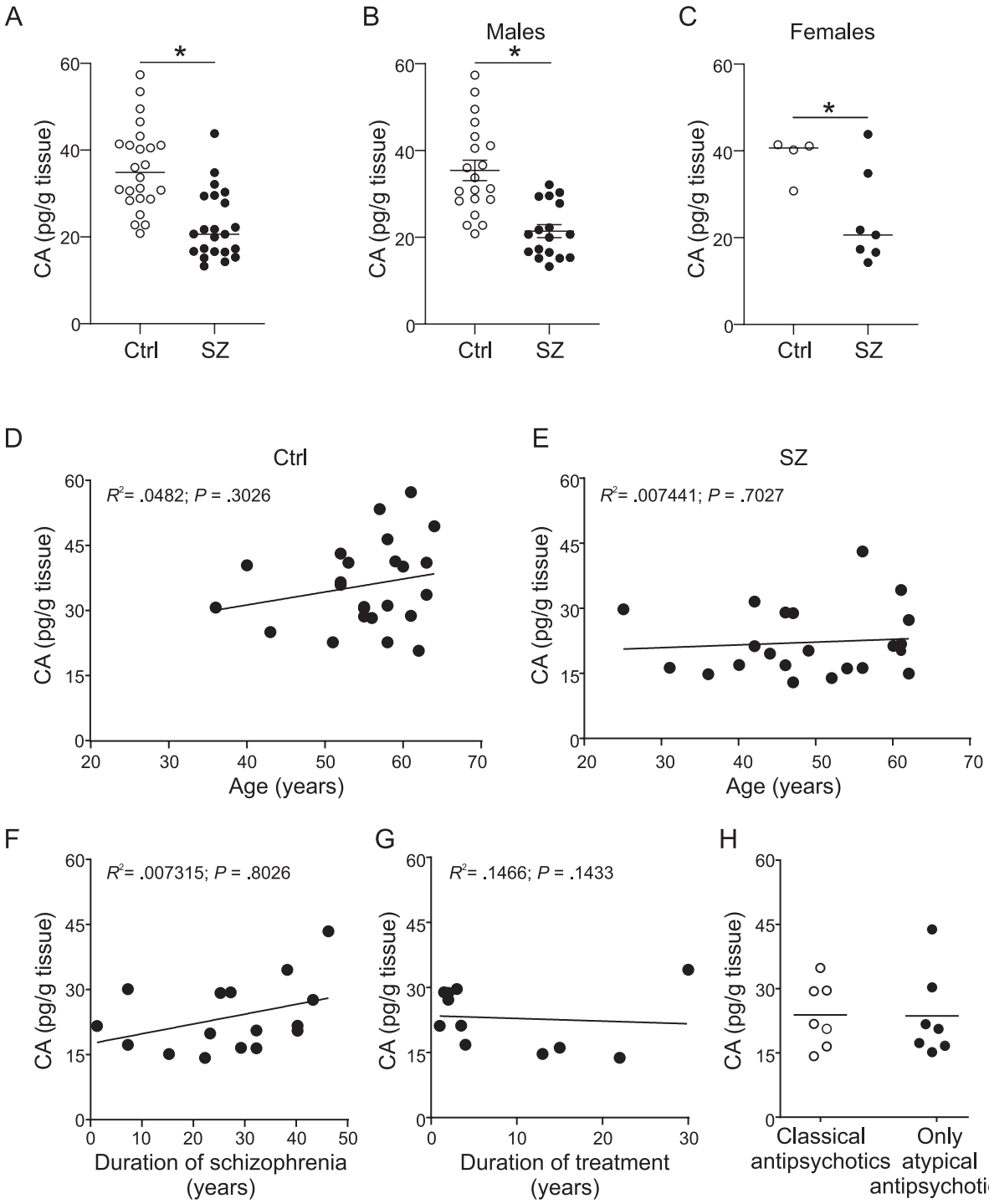

Fig. 1. Reduced endogenous cinnabarinic acid (CA) levels in PFC of individuals with schizophrenia. CA levels in prefrontal cortex samples from individuals with schizophrenia (SZ) and controls (Ctrl). One value in the SZ group (AN10924) and 2 values in the Ctrl group (AN01254 and AN02315, supplementary table S1) were identified as outlayers by the Grubbs' test and were excluded from the analysis. (A) Means ± S.E.M. are 35.92 ± 1.99 and 22.61 ± 1.69 (pg/g tissue) in Ctrl and SZ, respectively. *P = .0000084 (Student's t-test, t_{44} = 5.042). (B, C) Gender distribution of CA values *P < .05 (Student's t-test) vs Ctrl; t_{35} = 4.829 (B); t_9 = 2.405 (C). Lack of correlation between CA values and age (D, E), disease duration (F), or drug treatment duration (G). (H) CA values in SZ patients treated with classical antipsychotics (w/wo atypical antipsychotics) or with atypical antipsychotics only (supplementary table S1).

in a few samples and amyloid accumulation or tau pathology in 2 other samples. One individual with schizophrenia had neuropathological signs typical of multiple sclerosis. Several non-schizophrenic controls were under pharmacological treatment for cardiovascular disorders and diabetes mellitus. Postmortem intervals in this group ranged from 4.75 to 23.18 hours (supplementary table S1).

Combined analysis of CA levels in the 2 groups showed no correlation with postmortem intervals (supplementary figure S1), indicating the stability of CA levels. Moreover, the overall CA levels did not differ between males and females and did not correlate with age (not shown).

In the PFC of non-schizophrenic controls, endogenous levels of CA were 35.92 ± 1.99 pg/g tissue (mean ± S.E.M.), whereas they were significantly reduced (by 36%) in individuals with schizophrenia (figure 1A), and the reduction was not gender-dependent (figures 1B and

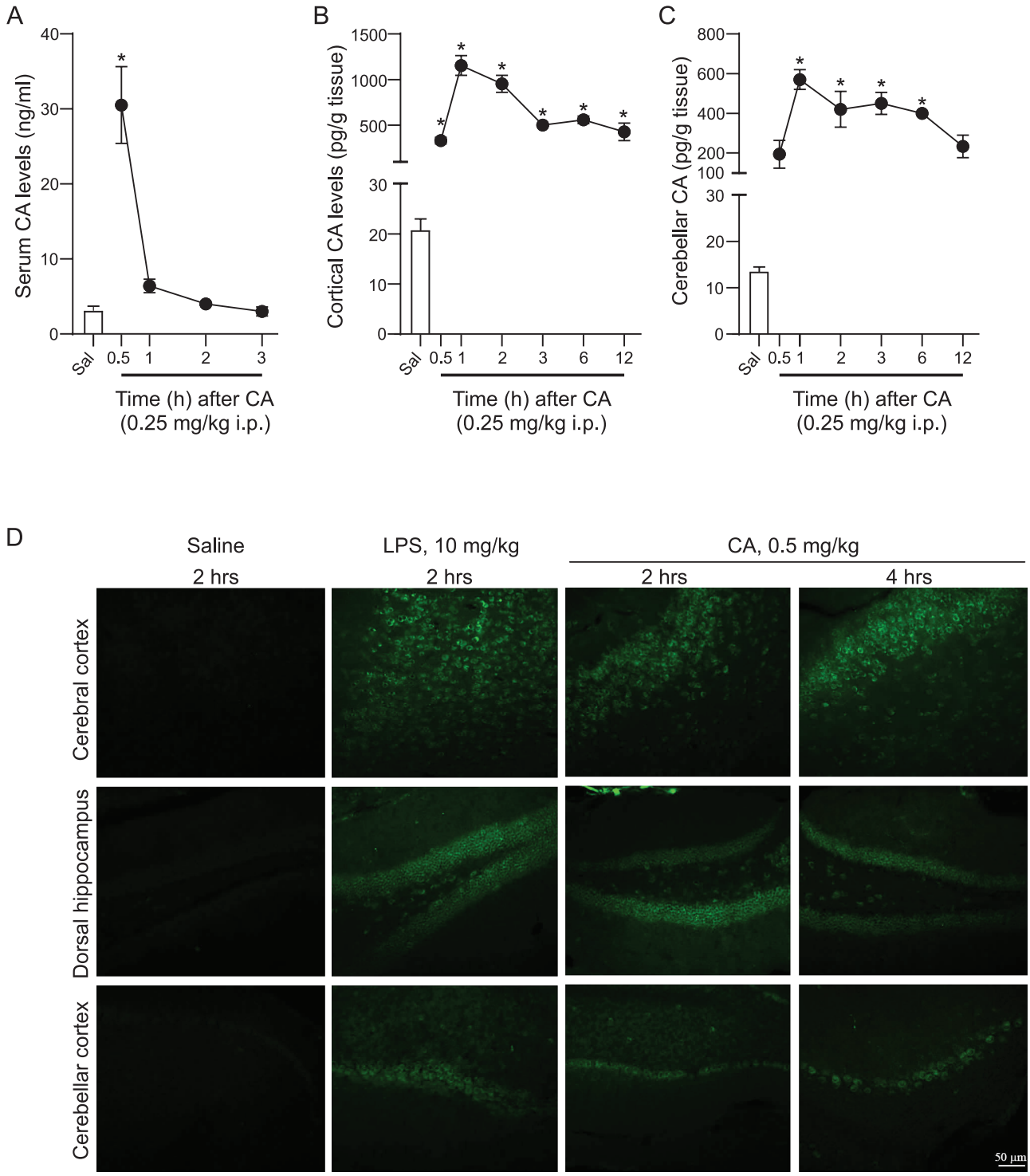

Fig. 2. Systemically administered cinnabarinic acid (CA) is brain-permeant. CA levels in serum, cerebral cortex, and cerebellum of mice treated with CA (0.25 mg/kg, i.p.) or saline are in (A), (B), and (C), respectively. Values are means ± S.E.M. (n = 2–3 mice per timepoint). *P < .05 (1-way ANOVA + Dunnet's test) vs saline; (A):[$F_{(4,7)}$ = 16.71]; (B):[$F_{(5,11)}$ = 36.902]; (C):[$F_{(6,10)}$ = 12.304]; (E) Immunohistochemistry in brain regions following i.p. injection of CA (0.5 mg/kg) or lipopolysaccharide (10 mg/kg).

1C). CA levels did not correlate with age in the 2 groups (figures 1D and 1E). In individuals with schizophrenia, PFC CA levels did not correlate with the duration of disease (figure 1F) and drug treatment (figure 1G). Finally, CA levels did not differ between the subgroups of individuals with schizophrenia treated with classical antipsychotics and atypical antipsychotics (figure 1H).

Behavioral and Biochemical Effects of CA Administration in Mice

Exogenous CA Is Brain-Permeant. We measured CA levels in mice receiving a single i.p. injection of low doses of CA (0.25 mg/kg) or saline. In mice treated with saline pg amounts of endogenous CA could be detected both in serum and brain tissue. Following CA injection, CA levels peaked after 30 minutes in serum and after 1 hour in the cerebral cortex and cerebellum (figures 2A and 2C). Levels remained high in brain tissue for 3 hours and declined afterwards (figures 2B and C). We confirmed that CA was brain-permeant by immunohistochemistry with a selective anti-CA antibody. Immunostaining became visible 2 and 4 hours after CA injection (figure 2D). Endogenous CA levels increased after treatment with LPS (10 mg/kg), which causes neuroinflammation (figure 2D).[40,41]

Low Doses of CA Are Effective in Behavioral Tests Predictive of Antipsychotic-Like Activity. Injection of MK-801

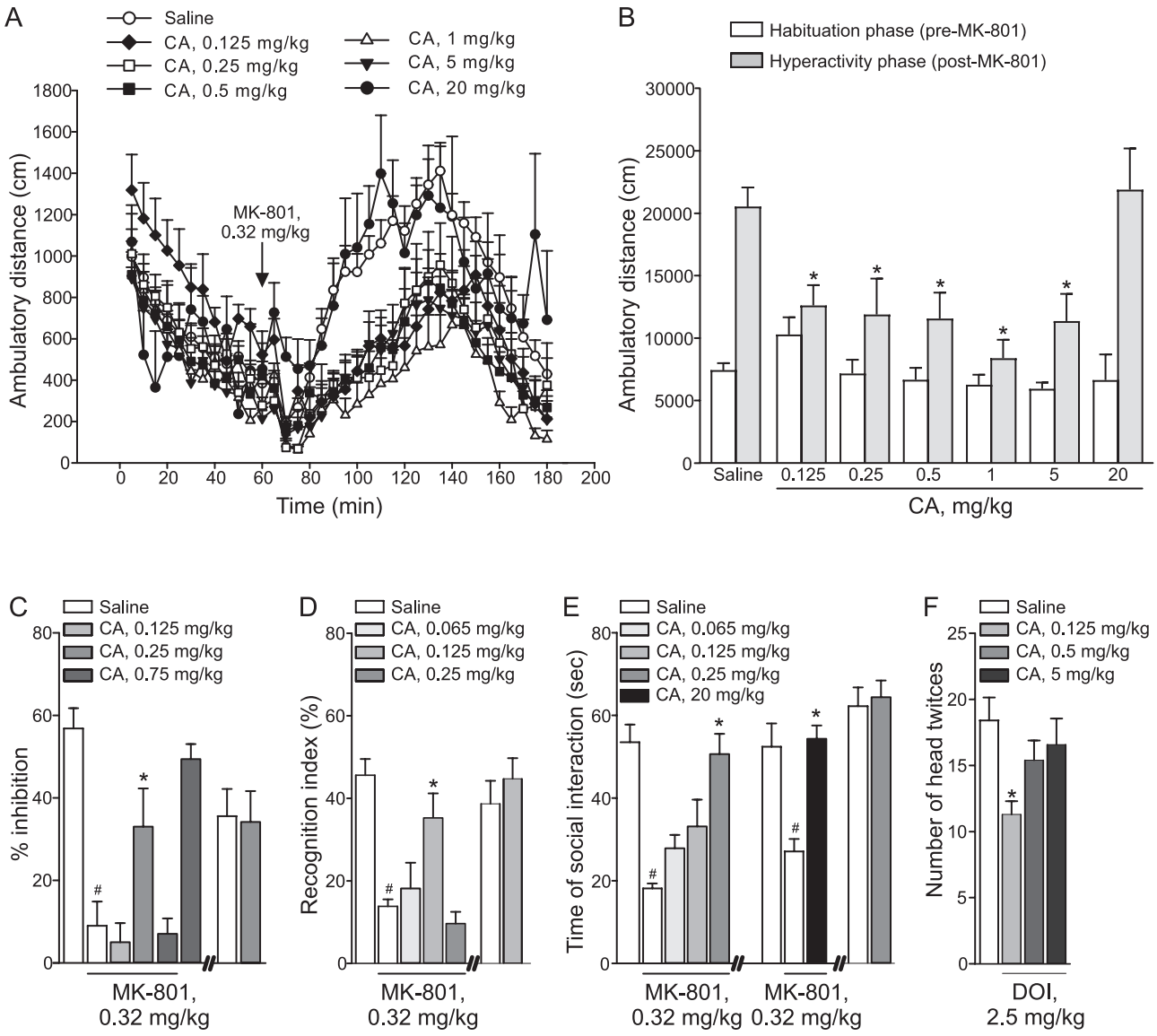

Fig. 3. Low doses of cinnabarinic acid (CA) display antipsychotic-like activity in rodents. MK-801 (0.32 mg/kg)-induced locomotor hyperactivity (A, B); MK-801-induced disruption of PPI (C), novel object recognition (D), and social interaction (E, F); DOI-induced head-twitches (G). Values are means ± S.E.M. (n = 6–29 in A and B; 10 in C; 8–10 in D–G). $P < .05$ vs saline (*) or MK-801 alone (#) (1-way ANOVA + Dunnett's test). (B) (hyperactivity phase):[$F_{(6,68)} = 5.920$]; (C):[$F_{(3,36)} = 4.47$]; (D):[$F_{(4,38)} = 14.17$]; (E):[$F_{(5,59)} = 16.822$]; (F):[$F_{(2,23)} = 15.903$]; (G):[$F_{(3,33)} = 4.061$].

(0.32 mg/kg, i.p.) in mice caused the expected escalation in locomotor activity. CA (0.125–20 mg/kg) or saline were administered i.p. 12 h prior to MK-801. CA had no effect by itself on locomotor activity (in the habituation phase) but largely reduced MK-801-induced hyperactivity at doses as low as 0.125 mg/kg. This action was maintained at doses ranging from 0.25 to 5 and lost at 20 mg/kg (figures 3A and 3B). In the PPI test, MK-801 boosted the amplitude of the acoustic startle response in rats and reduced PPI of the startle response by up to about 10% of controls. CA reversed the inhibitory action of MK-801 with an inverse U-shaped dose–response curve, being effective at the dose of 0.25 mg/kg, but not at 0.15 and 0.75 mg/kg (figure 3C). CA showed a similar dose-response curve in reversing MK-801-induced disruption of novel object recognition (figure 3D). In the social interaction test, the dose-response curve of CA differed with respect to all other tests. MK-801 significantly decreased the duration of social interactions and the number of episodes. CA inhibited the action of MK-801 in a dose-dependent manner between 0.065 and 0.25 mg/kg, an effect maintained at the dose of 20 mg/kg (figure 3E). In all these tests, CA had no activity in the absence of MK-801 (figures 3C and 3E). Low doses of CA (0.125 mg/kg) also reduced head-twitches induced by the 5-HT_{2A} receptor agonist,[42] DOI (2.5 mg/kg, i.p.) (figure 3F).

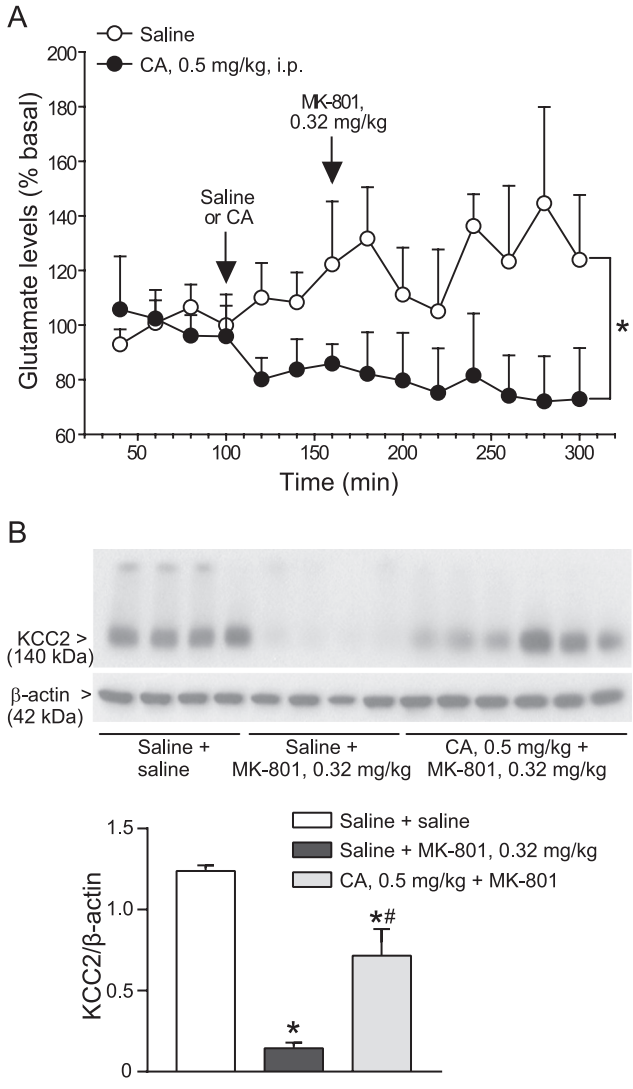

Fig. 4. Low doses of cinnabarinic acid (CA) correct MK-801-induced biochemical alterations in prefrontal cortex. (A) MK-801-evoked glutamate release in prefrontal cortex. Values are means ± S.E.M. (n = 7–8). *P < .05 (1-Way ANOVA for repeated measures + Fisher's test) [$F_{(1,69)}$ = 14.491]. (B) Immunoblot analysis of KCC2 in prefrontal cortex. Values are means ± S.E.M. (n = 4–6). P < .05 (1-way ANOVA + Fisher's test vs saline + saline [*] or saline + MK-801 [#]) [$F_{(2,11)}$ = 16.586].

Low Doses of CA Inhibit Biochemical Responses to MK-801. MK-801 is known to stimulate glutamate release[43–45] in the cerebral cortex and to reduce the expression of KCC2,[46,47] a K^+/Cl^- symport shaping synaptic responses mediated by $GABA_A$ receptors.[48,49] Both effects reflect a primary action of MK-801 at fast-spiking cortical interneurons, leading to disinhibition of pyramidal neurons.[13] In microdialysis studies, MK-801 (0.32 mg/kg, i.p.) enhanced glutamate release in PFC with a peakless effect lasting for at least 2 hours, an effect prevented by low doses of CA (0.5 mg/kg, i.p., 1 h prior MK-801) (figure 4A). CA also attenuated the lowering effect of MK-801 on KCC2 protein levels in the PFC (figure 4B).

Activation of mGlu4 Receptors Mediated the Antipsychotic-Like Activity of Low Doses of CA. Inhibition of MK-801-induced hyperactivity by low doses of CA in mice was not affected by treatment with the AhR antagonist, CH223191 (1 mg/kg), although a combination of CA and CH223191 reduced locomotor activity during the habituation phase (figures 5A and 5B). In contrast, low doses of CA (0.25–0.5 mg/kg) failed to inhibit MK-801-induced hyperactivity in $mGlu4^{-/-}$ mice (figures 5C and 5D), demonstrating that the antipsychotic-like activity of CA might be mediated by mGlu4 receptors.

In Vitro Studies

CA Has No Direct Effects on NMDA Receptors in Cultured Neurons. We used cultured cerebellar granule cells to exclude that CA could directly affect the inhibition of NMDA receptors by MK-801. Application of NMDA (100 µM) to cultures at 8 days in vitro caused a large increase in cGMP formation, which was abolished by MK-801 (1µM). CA (30–100 µM) did not affect any of these responses (supplementary figure S2).

Effect of CA on Excitatory Synaptic Transmission and Neurotransmitter Release. Low concentrations of CA (1 µM) depressed the frequency of excitatory synaptic transmission recorded in L5 pyramidal neurons in PFC slices prepared from wild-type mice, but not in slices

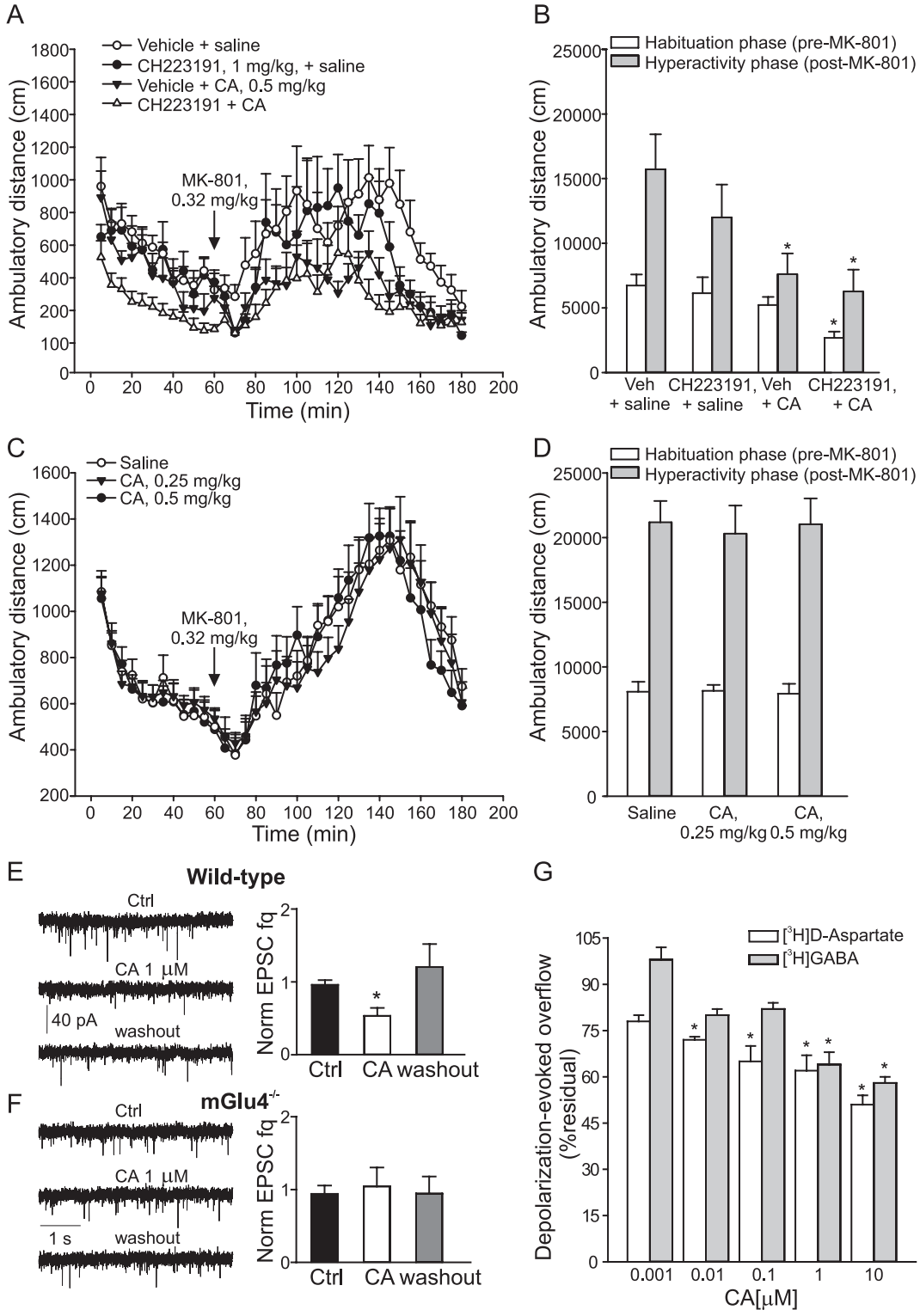

Fig. 5. mGlu4 receptors mediate behavioral and electrophysiological effects of cinnabarinic acid (CA). MK-801-induced hyperactivity in mice pretreated with CH223191 (A, B) or in mGlu4$^{-/-}$mice (C,D). Values are means ± S.E.M. (n = 7–8 in A and B and n = 15–16 in C and D). B, *P < .05 (1-way ANOVA + Dunnett's test) vs saline + vehicle (habituation phase:[$F_{(3,27)}$ = 4.4]; hyperactivity phase:[$F_{(3,27)}$ = 3.778]). Effect of CA (1 µM) on excitatory synaptic transmission in prefrontal cortex of wild-type (E) or mGlu4$^{-/-}$ (F) mice. Traces of EPSCs from L5 pyramidal neurons are shown. Values are means ± S.E.M. (n = 9 cells, 4 mice in E; n = 5 cells, 3 mice in F). *P < .05 (1-way ANOVA + Dunnett's test) vs controls. (G) Inhibition of depolarization-evoked [^{3}H]D-aspartate and [^{3}H]GABA release by CA in cortical synaptosomes. Results are percentage of K^+-evoked tritium overflow (% of residual). Values are means ± S.E.M. of 4 experiments in triplicate. *P < .05 (1-way ANOVA + Dunnett's test) vs tritium overflow in the absence of CA. ([^{3}H]D-aspartate:[$F_{(5,16)}$ = 6.103; [^{3}H]GABA:[$F_{(5,14)}$ = 4.302]).

prepared from mGlu4$^{-/-}$ mice (figures 5E and 5F). This suggested that low concentrations of CA inhibited excitatory amino acid release by activating presynaptic mGlu4 receptors.[50] Inhibition of glutamate release by low concentrations of CA was also demonstrated in superfused cortical synaptosomes preloaded with [^{3}H]D-aspartate (a non-metabolizable analog of glutamate). CA displayed a high potency in reducing depolarization-evoked [^{3}H]D-aspartate release, with an EC_{50} value as low as 3.82 ± 0.45 nM, and a maximal inhibition >40% at 10 μM (figure 5G). CA could also reduce [^{3}H]GABA release in cortical synaptosomes but with a much lower potency (EC_{50} value = 13.82 ± 4.1 μM).

Discussion

Using a highly sensitive UPLC/MS-MS method, we detected trace amounts of CA in human PFC. CA levels were substantially lower than those reported for other kynurenines in human brain tissue. For example, KYNA levels in the human cortex were approximately 2–3 pmoles (370–550 pg)/mg of protein, and L-kynurenine levels were 10-fold higher.[6,51] CA levels in the human PFC were as low as 0.02–0.04 pg/mg of wet tissue, corresponding to 0.2–0.4 pg/mg proteins if one assumes a 1:10 ratio between proteins and tissue weight. Similar amounts of endogenous CA were found in the brain tissue of mice (basal conditions). The trace amounts of CA found in brain tissue may cast doubts on the importance of CA in central nervous system (CNS) physiology and pathology. Nevertheless, we were surprised to find that very low doses of CA displayed antipsychotic-like activity in rodents. Remarkably, systemically administered CA was effective at doses of 0.125–0.5 mg/kg, which are at least 100-fold lower than those reported for other kynurenine metabolites in behavioral studies.[21,52,53] CA levels in the cerebral cortex increased for many hours after peripheral administration, indicating a low brain CA clearance. This suggests that CA itself rather than a peripheral metabolite was behaviorally active.

Noteworthy, the potency of CA in some behavioral tests was not homogeneous. For example, relatively high doses of CA (20 mg/kg) lost efficacy in reducing hyperactivity but were active in the social interaction test. Moreover, the action of CA in PPI and novel object recognition tests emerged only at very low doses (0.25–0.125 mg/kg). These differences might be related to off-target effects produced by increasing doses of CA, which may differentially affect individual behavioral tests. The "therapeutic effect" of low doses of CA in the PPI test extends the interest for CA to CNS disorders other than schizophrenia, such as obsessive-compulsive disorder, Tourette's syndrome, and posttraumatic stress disorder.[54]

Data obtained in mGlu4$^{-/-}$ mice suggest that at least the action of CA on MK-801-induced hyperactivity and excitatory synaptic transmission in L5 pyramidal neurons was mediated by mGlu4 receptors. These receptors are localized in presynaptic terminals and inhibit glutamate release.[50] We were intrigued by the finding that CA inhibited excitatory synaptic transmission in the PFC and excitatory amino acid release in cortical synaptosomes at concentrations lower than those required to activate mGlu4 receptors in heterologous expression systems.[22] Perhaps the interaction of mGlu4 receptors with adaptor or scaffolding proteins in its native environment enhances the potency of CA as an orthosteric agonist. This hypothesis warrants further investigation.

Although endogenous CA levels were low in the human PFC, they were largely reduced in samples from individuals with schizophrenia. Notably, CA levels did not correlate with postmortem intervals, suggesting that CA is relatively stable in brain tissue. The lack of correlation with disease duration and treatment suggests that a reduced formation (or an increased clearance) of CA in the PFC is not secondary to antipsychotic medication. The defective KMO activity associated with schizophrenia (see Introduction and References therein) might contribute to the reduced formation of CA, similarly to what observed with other kynurenine metabolites that lie downstream of 3-hydroxykynurenine.[21] Whether the formation of CA from 3-HANA is defective in schizophrenia remains to be determined. Interestingly, CA displays anti-inflammatory activity by activating mGlu4 receptors and *via* other mechanisms.[55–57] The tight association between neuroinflammation and schizophrenia[29–33] suggests that the protective role of CA in schizophrenia may extend beyond the control of synaptic transmission and involves other mechanisms laying at the core of the disorder.

In conclusion, our findings suggest that the trace kynurenine, CA, is a potential new player in mechanisms that contribute to the pathophysiology of schizophrenia and raise the attractive possibility that even low doses of CA may improve psychotic symptoms and slow the progression of the disease by attenuating the hyperactivity of pyramidal neurons and restraining neuroinflammation (supplementary figure S3). If safe and tolerated, low doses of CA could be administered to patients with schizophrenia in an attempt to reduce psychotic symptoms and restrain neuroinflammation associated with the disease.

Supplementary Material

Supplementary material is available at *Schizophrenia Bulletin* online.

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