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Research Paper

Quercetin Reduces Cortical GABAergic Transmission and Alleviates MK-801-Induced Hyperactivity

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ABSTRACT

An imbalance between neuronal excitation and inhibition represents a core feature in multiple neuropsychiatry disorders, necessitating the development of novel strategies to calibrate the excitatory–inhibitory balance of therapeutics. Here we identify a natural compound quercetin that reduces prefrontal cortical GABAergic transmission and alleviates the hyperactivity induced by glutamatergic *N*-methyl-D-aspartate receptor antagonist MK-801. Quercetin markedly reduced the GABA-activated currents in a noncompetitive manner in cultured cortical neurons, and moderately inhibited spontaneous and electrically-evoked GABAergic inhibitory postsynaptic current in mouse prefrontal cortical slices. Notably, systemic and prefrontal-specific delivery of quercetin reduced basal locomotor activity in addition to alleviated the MK-801-induced hyperactivity. The effects of quercetin were not exclusively dependent on α5-subunit-containing A type GABA receptors ($GABA_ARs$), as viral-mediated, region-specific genetic knockdown of the α5-subunit in prefrontal cortex improved the MK-801-evoked psychotic symptom but reserved the pharmacological responsivity to quercetin. Both interventions together completely normalized the locomotor activity. Together, quercetin as a negative allosteric $GABA_AR$ modulator exerted antipsychotic activity, facilitating further therapeutic development for the excitatory–inhibitory imbalance disorders.

1. Introduction

Accurate calibration of excitatory–inhibitory balance across the central nervous system is fundamental for the normal functioning of the brain, while an imbalance in neuronal excitation/inhibition is a core feature observed in neuropsychiatric disorders, but not restricted to schizophrenia. The finding [37] that the psychotomimetic drug phencyclidine noncompetitively blocked the *N*-methyl-D-aspartate receptor (NMDAR) gave rise to the glutamate theory of schizophrenia [10, 11, 26], according to which NMDAR hypofunction and disturbances in NMDAR-related gene expression and metabolic pathways confer the disease phenotypes [46, 62]. The NMDAR antagonist ketamine has been shown to induce significant psychosis [31] and exacerbated it further in individuals predisposed to schizophrenia symptomatology [32]. Consequently, the NMDAR antagonist such as MK-801 has been shown to induce hyperactivity using locomotor activity paradigm in rodents to model the part of the positive symptoms in psychosis [1, 23, 70, 71] for novel antipsychotic drug discovery [58] and therapeutic development [42–44]. Not surprisingly, agents potentiating glutamatergic transmission, by activating the glycine modulatory site on the NMDAR, have

Abbreviations: AAV, Adeno-associated virus; ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DAPI, 4,6-diamidino-2-phenylindole dihydrochloride; D-APV, D-2-amino-5-phosphonopentanoic acid; DMSO, dimethyl sulfoxide; EGTA, ethylene glycol tetraacetic acid; eEPSC, evoked excitatory postsynaptic current; eIPSC, evoked inhibitory postsynaptic current; EPSC, excitatory postsynaptic current; EYFP, enhanced yellow fluorescent protein; GABA, γ-aminobutyric acid; $GABA_AR$, A-type GABA receptor; $GABA_CR$, C-type GABA receptor; GFP, green fluorescence protein; HEPES, *N*-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid; i.p., intraperitoneal; IPSC, inhibitory postsynaptic current; mPFC, medial prefrontal cortex; NC-Ctrl, negative control; n_H, Hill coefficient; NMDA, *N*-methyl-D-aspartate; NMDAR, *N*-methyl-D-aspartate receptor; PBS, phosphate-buffered solution; sIPSC, spontaneous inhibitory postsynaptic current.

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been reported to reduce some of the cognitive symptoms of schizophrenia [4, 14, 15]. Specifically, schizophrenia, particularly the cognitive symptoms of the disorder, may result from the low activity of NMDAR on the GABAergic inhibitory interneurons in the prefrontal cortex [10–12, 26, 42, 43, 46, 71], as the postnatal ablation of NMDAR in this subtype of neurons conferred most schizophrenia-like phenotypes [5, 20]. The progress in the understanding of pathogenesis of psychosis is encouraging; however, unfortunately, current treatments for schizophrenia are far from satisfactory. Yet, these treatments have substantially improved outcomes for most patients with schizophrenia [26]. Therefore, continued efforts are necessary to develop or discover novel strategies for preventing and curing this type of disorder.

The γ-aminobutyric acid (GABA) system is pivotal for the orchestration of local networks and the functional interaction across different brain regions [64], which can act as an alternative target to calibrate the excitatory–inhibitory balance in the central nervous system. A-type GABA receptors ($GABA_AR$s) are pentameric Cl^--permeable ion channels activated by the GABA transmitter and widely distributed in the central nervous system, which primarily confer fast inhibitory control over neural activity [56]. To date, at least up to 19 known subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3) have been identified. Of note, many of functional $GABA_AR$s contain two α-subunits, two β-subunits, and one γ-subunit [41, 55]. Because $GABA_AR$s are responsible for inhibitory tone in the central nervous system, they are indispensable for controlling the neuronal balance between excitation and inhibition and thus participate in almost every physiological and pathophysiological brain function. Accordingly, $GABA_AR$s have long been considered as an important pharmaceutical target. They are positively modulated [59] by benzodiazepines [63], barbiturates [38], steroids [25], and anesthetics [45, 52, 65, 69]. Most of these drugs have been in clinical use for decades and are still among the most widely prescribed drugs for the treating insomnia and anxiety disorders.

Growing evidence also suggests that low doses of $GABA_AR$ antagonists show therapeutic potentials in a particular type of neurodevelopmental disorders such as in Down syndrome [18] and the antipsychotic efficacy [51, 60]. Of note, negative allosteric modulators selectively targeting α5-subunit-containing $GABA_AR$s consistently exhibit substantial pharmacological effects to restore cognitive deficits [2, 3, 30, 40, 53, 61], promote functional recovery after stroke [8], or exert the anti-depressant action [72]. It is worth noting that $GABA_AR$-mediated inhibition can be cell type-specific [66], and targeting $GABA_AR$s would have different roles in the network dependent on the target neurons [17]. Inhibiting glutamatergic pyramidal neuron by $GABA_AR$ reduces network excitability while inhibiting GABAergic interneurons increases network excitability. Nevertheless, most of GABAergic agents do not distinguish between these two alternatives, providing an alternative explanation for the aforementioned $GABA_AR$ inhibitors, on occasion, capable of reducing network excitability for their beneficial efficacy. Collectively, therapeutic potentials by virtue of negative modulators of $GABA_AR$s for neuropsychiatric disorders [6, 29, 64], including schizophrenia, are far underestimated.

The current study took advantage of a natural flavonoid compound quercetin [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one, Fig. 1A], which has been identified as a negative modulator for recombinant $GABA_AR$s and C-type GABA receptors ($GABA_CR$s, also designated to be ρ1-subunit-containing $GABA_AR$s) [21, 22, 28], to examine its pharmacological effects on brain activity. The present study examined the effects of quercetin on the endogenous $GABA_AR$ currents in cultured mouse cortical neurons, in addition to that on synaptic transmission in mouse prefrontal cortex slices (using patch-clamp electrophysiology), and MK-801-evokded locomotor hyperactivity (using behavioral assessment). The study found that quercetin, as an inhibitor of $GABA_AR$s, reduced GABAergic transmission in the prefrontal cortex and alleviated the hyperactivity caused by MK-801.

2. Materials and Methods

2.1. Animals

All behavioral measurements were performed in adult unrestrained awake male C57BL/6J mice (8–12 weeks old), which were obtained from Shanghai Slac Laboratory Animal Company Limited (Shanghai, China). The Mice were subjected to a 12-h light/dark cycle, and the behavioral experiments were always performed during the light phase of the cycle. The mice had access to food and water *ad libitum* except during tests. All efforts were made to minimize animal suffering and reduce the number of animals used. All experimental protocols were approved by the Animal Ethics Committee of Shanghai Jiao Tong University School of Medicine, China. In all experiments, the investigators were blind to the drug treatment of mice. The experiments were performed on the mice in a randomized order.

2.2. Drugs

Primary cultures of mouse cortical neurons were prepared according to previously described techniques [36]. In brief, 15-day-old embryonic C57BL/6 J mice were isolated using a standard enzyme treatment protocol. Brains were removed rapidly and placed in ice-cold Ca^{2+}- and Mg^{2+}-free phosphate-buffered solution (PBS). Tissues were dissected and incubated with 0.05% trypsin-EDTA for 10 min at 37 °C, followed by trituration with fire-polished glass pipettes, and plated on poly-D-lysine-coated 35 mm culture dishes (Corning, USA) at a density of 1×10^6 cells per dish. Neurons were cultured using Neurobasal medium (Thermo Fisher Scientific, USA) supplemented with B27 (Thermo Fisher Scientific, USA) and maintained at 37 °C in a humidified 5% CO_2 atmosphere incubator. Cultures were fed twice a week and used for electrophysiological recording 10–20 days after plating.

The cDNA of mouse $GABA_AR$ α5-subunit (GenBank accession: NM_176942.4) or γ2-subunit (GenBank accession: NM_008073.4) was expressed in human embryonic kidney (HEK)-293T cells by transient transfection as reported in a previous study [36]. The HEK-293T cells were cultured in the Dulbecco's modified Eagle's medium supplemented with 1 mM L-glutamine, 10% fetal bovine serum, 50 units/ml penicillin, and 50 μg/ml streptomycin (all from Thermo Fisher Scientific, USA), at 37 °C in a humidified atmosphere of 5% (v/v) CO_2 and 95% O_2 (v/v) and passaged twice a week. Transient transfection of HEK-293T cells was performed using HilyMax liposome transfection reagent (Dojindo Laboratories, Japan).

2.3. Chemicals

All drugs were purchased from Sigma-Aldrich (Merck Millipore, USA) except otherwise indicated. In the electrophysiological experiment, the final concentration of dimethyl sulfoxide (DMSO) was lower than 0.1% and verified to be ineffective alone at the same concentration in control experiment. Other drugs were first dissolved in ion-free water and then diluted to the final concentrations in the standard external solution just before use or dissolved directly in the standard external solution.

2.4. Electrophysiological Recording in Cultured Cells

Whole-cell recordings were made using an Axon 200B patch-clamp amplifier (Axon Instruments, USA). Membrane currents were sampled and analyzed using a Digidata 1440 interface and a personal computer running Clampex and Clampfit software (Version 10, Axon Instruments). The membrane potential was held at −60 mV throughout the experiment under voltage clamp conditions. All the experiments were carried out at room temperature (23 ± 2 °C).

The standard external solution contained (in mM): 150 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 *N*-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid

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