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Acute and Chronic Effects of Clozapine on Cholinergic Transmission in Cultured Mouse Superior Cervical Ganglion Neurons

Taixiang Saur,

McLean Hospital/Harvard Medical School, 115 Mill St., Belmont, MA, 02478, USA.

Bruce M. Cohen,

McLean Hospital/Harvard Medical School, 115 Mill St., Belmont, MA, 02478, USA.

Qi Ma,

New England Primate Research Center, Harvard Medical School, Southborough, MA 01772

Present address: Departments of Psychiatry and Neuroscience, Upstate Medical University, State University of New York, 505 Irving Ave, Syracuse, NY 13210, USA.

Suzann M. Babb,

McLean Hospital/Harvard Medical School, 115 Mill St., Belmont, MA, 02478, USA.

Edgar A. Buttner, and

McLean Hospital/Harvard Medical School, 115 Mill St., Belmont, MA, 02478, USA.

Wei-Dong Yao

New England Primate Research Center, Harvard Medical School, Southborough, MA 01772

Present address: Departments of Psychiatry and Neuroscience, Upstate Medical University, State University of New York, 505 Irving Ave, Syracuse, NY 13210, USA.

Abstract

Cholinergic dysfunction contributes to cognitive deficits associated with various neuropsychiatric disorders, including schizophrenia. The atypical antipsychotic clozapine improves cognitive function in patients with schizophrenia, possibly through modulation of the cholinergic system. However, little is known about specifics of the underlying mechanisms. In this study, we investigated the acute and chronic effects of clozapine on cholinergic synaptic transmission in cultured superior cervical ganglion (SCG) neurons. Spontaneous excitatory postsynaptic currents (sEPSCs) were readily detected in this preparation and were reversibly inhibited by the nicotinic receptor antagonist d-tubocurarine, confirming that the synaptic responses were primarily mediated by nicotinic receptors. Bath application of clozapine at therapeutic concentrations rapidly and reversibly inhibited both the amplitude and frequency of sEPSCs in a concentration dependent manner, without changing either rise or decay time, suggesting that clozapine effects have both presynaptic and postsynaptic origins. The acute effects of clozapine on sEPSCs were recapitulated by chronic treatment of SCG cultures with similar concentrations of clozapine, as clozapine treatment for four days reduced the frequency and amplitude of sEPSCs without

affecting their kinetics. Cell survival analysis indicated that SCG neuron cell counts after chronic clozapine treatment were comparable to the control group. These results demonstrate that therapeutic concentrations of clozapine suppress nicotinic synaptic transmission in SCG cholinergic synapses, a simple in vitro preparation of cholinergic transmission.

Keywords

clozapine; cholinergic transmission; sEPSCs; nicotinic receptors; superior cervical ganglion; atypical antipsychotic drugs

Introduction

Acetylcholine (ACh) has long been known to play a role in various domains of normal cognition (Deutsch, 1971; Sarter & Parikh, 2005). Cholinergic dysfunction is central to the pathophysiology of Alzheimer's disease (Cummings & Benson, 1987; Schliebs & Arendt, 2011) and has also been postulated to contribute to the cognitive deficits of various neuropsychiatric disorders, notably including schizophrenia (Featherstone & Siegel, 2015; Gibbons & Dean, 2016; Ochoa & Lasalde-Dominicci, 2007; Ripoll et al., 2004). In particular, recent studies in humans have underscored the potential importance of nicotinic acetylcholine receptors (nAChRs) in the pathophysiology of schizophrenia owing to: (1) the abnormal expression of nAChRs in post-mortem brains of schizophrenic patients; (2) the striking association between smoking and schizophrenia; (3) the improved cognition seen in animals and humans with nicotine exposure; (4) the genetic association between the human $\alpha 7$ -nAChR and schizophrenia; and (5) the potential efficacy of nAChR agonists as treatments for cognitive and emotional deficits in schizophrenia (Freedman et al., 2008; Harrison & Weinberger, 2005; Martin & Freedman, 2007).

Clozapine was the first atypical antipsychotic drug (APD) shown to be effective in treating the cognitive dysfunction of schizophrenia and remains the only drug with strong evidence for ameliorative effects on cognition (Hagger et al., 1993). Although APDs all act in part by inhibiting dopamine receptors, it is also widely recognized that their therapeutic efficiency is related to their target diversity, which includes numerous receptors and ion channels (Stahl, 1999), including neuronal nicotinic receptors. The electrophysiological evidence supports direct interactions between nicotinic receptors and APDs, which often inhibit nAChRs. For example, clozapine inhibits nAChRs in bovine adrenal chromaffin cells, leading to the subsequent inhibition of catecholamine secretion (Park et al., 2001). Clozapine also inhibits the $\alpha 1\beta 1\gamma\delta$ subunit combination of the embryonic nAChR expressed in *Xenopus* oocytes (Nguyen & Miledi, 2002). The frog muscle neuromuscular junction has been a useful preparation to study nicotinic transmission, and APDs have been reported to inhibit the muscle nAChRs (Nguyen & Miledi, 2002). In mammals, clozapine inhibits the function of $\alpha 7$ -nAChRs in hippocampal neuronal preparations (Singhal et al., 2007). Similarly, APDs non-competitively inhibit the function of neuronal $\alpha 4\beta 2$ and $\alpha 7$ nicotinic receptors (Grinevich et al., 2009). In vivo animal studies, however, show that APDs preferentially increase the release of ACh in the cortex and hippocampus, and the mechanisms possibly involve indirect effects through the interactions with 5-HT_{1A}, 5-HT_{2A}, dopamine D₂

receptors, $\alpha 1/2$ adrenoceptors and muscarinic M2 receptors (Chung et al., 2004; Ichikawa et al., 2002; Johnson et al., 2005; Shirazi-Southall et al., 2002).

The cholinergic synapse formed between superior cervical ganglion (SCG) neurons in long-term culture is a useful cellular model for studying fast cholinergic transmission and modulation as it can reveal both the presynaptic and postsynaptic mechanisms underlying physiologic changes (Luther et al., 2013; Ma & Mochida, 2007; Mochida et al., 1994; Nai et al., 2010). In this study, we employed this system and investigated acute and long-term effects of clozapine on cholinergic transmission. We observed that concentrations of clozapine achieved during therapeutic use produced concentration-dependent decreases in both the amplitude and frequency of nAChR-mediated sEPSCs, without affecting the kinetics of the cholinergic response, suggesting that clozapine inhibited nicotinic synaptic transmission via both presynaptic and postsynaptic mechanisms. The results may help advance understanding of the clinically significant impact of clozapine on cognitive function through its modulation of nicotinic transmission.

Materials and Methods

SCG cell culture

Mice were sacrificed by CO₂ exposure according to National Institutes of Health guidelines for the care and use of animals and with an approved animal protocol from the Harvard Medical Area Standing Committee on Animals. SCGs were dissected from postnatal 2–5 day mice on the C57BL6 genetic background. The ganglia from 5–10 animals were pooled and kept in ice-cold Hank's balanced salt buffer (HBSS, Invitrogen) and digested with 0.4% trypsin 100ul solution plus 50 μ l deoxyribonuclease solution (Worthington Biochemical) for 45 min at 37°C. Neurons were dispersed by trituration and centrifuged at 500g for 5 min at room temperature. After removing the supernatant, the tissue was re-suspended in 100 μ l ovomucoid inhibitor-albumin solution plus 25 μ l deoxyribonuclease solution (Worthington Biochemical) and centrifuged at 500g for 5 min. The tissue was then re-suspended and centrifuged in Sterile Earle's balanced Salt Solution (EBSS) (Worthington Biochemical) twice.

Neurons were then re-suspended in culture medium containing: neurobasal medium (catalog 21103–049, Invitrogen) with 1X B-27 supplement (catalog, Invitrogen), 1X GlutaMAX-I (catalog 35050–061, Invitrogen), 1% penicillin and streptomycin (catalog 15140–122, Invitrogen), 10% FBS, and 50 ng/ml NGF2.5 S (catalog 13257–019, Invitrogen). 500 μ l culture medium was half-changed (250 μ l old medium was replaced with 250 μ l fresh medium) twice a week. The plated cell density was about one ganglion per well. The culture was kept in a 37°C incubator supplied with 5% CO₂ and 95% O₂. SCG cells developed without NGF generally did not grow neurites and did not survive to DIV7, consistent with the requirement for NGF in the survival and neurite growth of these neuronal cultures (Blennerhassett & Bienenstock, 1998). In the presence of NGF, SCG neurons started to extend processes at DIV4 and their round somata enlarged (10–20 μ m diameter) and the neurites formed complex connections around DIV14 (cf. Fig. 3A). SCG cells can survive more than one month. Chronic treatment with clozapine started on day-in-vitro (DIV) 10

and was maintained for 4 days. Different concentrations of clozapine were dissolved in DMSO with a final concentration of 0.1% in culture medium.

Surviving rates were measured 4 days after different treatments by counting cells randomly in 5 different fields under the microscope at 10X magnification for each cover slip. At least 3 cover slips were counted for each treatment from the same culture group. All quantification data obtained from 3 independent repeats were averaged and normalized to the control condition, treated with 0.1% DMSO.

Electrophysiology

Whole-cell voltage-clamp recordings were made with an Axoclamp 700B amplifier (Molecular Devices) at room temperature. Neurons were perfused continuously throughout the recording with an external solution consisting of (in mM): NaCl, 145; KCl, 3; MgCl₂, 1; CaCl₂, 2; glucose, 10; N-2-hydroxyethyl piperazine-N0-2-ethanesulfonic acid (HEPES), 10; pH 7.3 adjusted with NaOH. Patch electrodes were pulled by a P-97 (Sutter Instrument) with a resistance of 2–4 MΩ. The electrodes were filled with the following (in mM): 142 Cs-gluconate, 8 NaCl, 10 HEPES, 0.4 EGTA, 2.5 QX-314 [*N*-(2,6-dimethylphenyl)carbamoylmethyl] triethylammonium bromide], 2 Mg-ATP, and 0.25 GTP-Tris, pH 7.25 (with CsOH). Membrane currents were filtered at 1.0 kHz sampled at 5 kHz. Drugs were delivered to the bath with a gravity-driven perfusion system (Harvard Apparatus). Neurons were voltage clamped at –60 mV. Seal resistance was monitored throughout the experiments. All experiments were performed using neurons cultured for 2–4 weeks. For chronic treatment, clozapine was present for four days and ciliary neurotrophic factor (CNTF, 20 ng/ml) was added on DIV1, and both were replaced by control external saline before recordings. sEPSCs were recorded by pClamp 10.0 (Molecular Devices) and analyzed by Mini Analysis 6 (Synaptosoft). Individual sEPSCs were inspected manually to eliminate false events.

Drugs used and their sources were as follows: clozapine (sigma), D-(–)-2-amino-5-phosphonopentanoic acid (APV) (Sigma), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Sigma), CNTF (Alomone), d-tubocurarine (d-TC) (Sigma), QX-314 (Sigma). Values are expressed as mean ± SEM. Statistical analysis was performed using Student's *t*-tests or one-way ANOVA followed by *posthoc* Tukey–Kramer tests, as appropriate.

Results

Cholinergic synaptic activity in cultured SCG cells

We recorded synaptic events from cultured SCG neurons under whole-cell voltage-clamp configuration at –60 mV. These events were detected by DIV6–7, but their frequency and amplitude were negligible, and the incidence of recording sEPSCs was low. Thus, data presented in this study were obtained from DIV14 or older. Results from neurons at around DIV14 showed no significant difference from neurons at around DIV28 (data not shown). sEPSCs displayed a low frequency, ranging from 0.01 to 1.02 Hz (Fig. 1). 35 of 116 neurons (30.2%) displayed a very low frequency (< 0.1 Hz) and were excluded from subsequent data analysis. Ciliary neurotrophic factor (CNTF) has been shown to promote cholinergic

transmission in cultured rat SCG neurons (Nai et al., 2010). Indeed, in our study, SCG cells cultured in a medium supplemented with CNTF (20 ng/ml) displayed a higher frequency (0.70 ± 0.08 Hz, $p < 0.01$ vs 0.28 ± 0.06 of control, Student's t test) and larger amplitude in sEPSCs (567.1 ± 60.5 pA, $p < 0.05$ vs. 328.6 ± 48.3 pA of control, Student's t test). In addition, all neurons treated with CNTF exhibited sEPSCs.

The transmitter for synapses between SCG neuron changes from noradrenaline (Koh & Hille, 1997) to acetylcholine in long-term culture (Mochida et al., 1994). To ensure that the sEPSCs we recorded were cholinergic, we applied the specific nicotinic receptor antagonist d-TC (100 μ M) to the extracellular bath. d-TC markedly suppressed sEPSCs (Fig. 1A). The frequency (0.05 ± 0.02 Hz, $p < 0.01$ VS 0.29 ± 0.04 Hz of control group, ANOVA; $n = 4$; Fig. 1C) and amplitude (48.4 ± 18.9 pA, $p < 0.01$ VS 288.7 ± 78.8 pA of control, ANOVA; Fig. 1B) of the sEPSCs were significantly decreased by d-TC, indicating that the sEPSCs in our cultured SCG neurons were mainly mediated by nicotinic receptors. Washing out d-TC recovered both the amplitude and frequency of sEPSCs (Fig. 1A-C). In contrast, the rise and decay times of sEPSCs remained unchanged following d-TC treatments and after d-TC washout (Fig. 1D, E). Thus, SCG neurons in culture represent an in vitro system for electrophysiological investigation of cholinergic synaptic transmission.

Acute clozapine treatment inhibits sEPSCs

To investigate the acute effects of clozapine on cholinergic transmission in SCG neurons, clozapine at a range of concentrations (dissolved in 0.1% DMSO) was added to the perfusion solution after recording baseline sEPSCs for several minutes. Bath application of 0.5 μ M or 2 μ M clozapine markedly and reversibly inhibited the amplitude (Fig. 2A, B and C) and the frequency (Fig. 2A, B and D), but had no effect on the rise time (Fig. 2E) and the decay time (Fig. 2F) of sEPSCs. The inhibition of sEPSCs amplitude was concentration-dependent. The amplitude decreased from 365.9 ± 79.3 pA (control) to 221.9 ± 42.6 pA at the concentration of 0.5 μ M ($p < 0.05$, ANOVA; $n = 5$) and 341.3 ± 73.3 pA to 125.9 ± 34.1 pA at 2 μ M ($p < 0.01$, ANOVA; $n = 5$) (Fig. 2C). The frequency decreased from 0.33 ± 0.04 Hz in control group to 0.23 ± 0.04 Hz at 0.5 μ M ($p < 0.05$, ANOVA) and from 0.30 ± 0.07 Hz to 0.18 ± 0.05 Hz at 2 μ M ($p < 0.05$, ANOVA) (Fig. 2D). The inhibition of both sEPSC frequency and amplitude by clozapine suggests that clozapine acts on both presynaptic release mechanisms and postsynaptic receptor responsiveness.

Chronic clozapine treatment suppresses sEPSCs

To investigate the long-term effects of clozapine on cholinergic transmission in SCG neurons, we treated cultures with clozapine at 0.5 μ M, 2 μ M or 0.1% DMSO (as control) starting at DIV10 for 4 days. The images of cells shown in Fig. 3A-C were taken four days after clozapine or DMSO treatment. To determine whether clozapine affects SCG neuronal survival, cells were counted in different fields under the microscope at 10X magnification and at least three coverslips were examined four days after treatment. Three independent experimental repeats were averaged and normalized. No significant difference in cell survival was found after treatment with 0.5 (normalized count 1.09 ± 0.05 , $p > 0.05$, ANOVA) or 2 μ M clozapine (normalized count 0.95 ± 0.05 , $p > 0.05$, ANOVA; Fig. 3D).

We next investigated the effects of chronic clozapine treatment on sEPSCs. In 0.5 μ M clozapine-treated cultures, the amplitude of sEPSCs was modestly attenuated (277.4 ± 75.2 pA vs. 354.9 ± 70.8 pA of control, $p > 0.05$, ANOVA) but the attenuation did not reach statistical significance (Fig. 4B). 0.5 μ M clozapine also did not affect the frequency significantly (0.21 ± 0.05 Hz vs. 0.37 ± 0.10 Hz of control, $p > 0.05$, ANOVA; Fig. 4C). However, 4-day treatment with 2 μ M clozapine significantly decreased the sEPSC amplitude to 214.9 ± 47.9 pA ($p < 0.05$, ANOVA) (Fig. 4B). The frequency of sEPSCs was also markedly inhibited by 2 μ M clozapine, from 0.37 ± 0.10 Hz to 0.12 ± 0.03 Hz in 2 μ M clozapine-treated neurons ($p < 0.05$, ANOVA; Fig. 4C). Similar to the results with acute treatment of clozapine, the rise time and decay time of sEPSCs were not affected by chronic treatment with clozapine (Fig. 4D and E),

Discussion

In this study, we observed that fast excitatory transmission in cultured SCG neurons is mainly mediated by nicotinic receptors, as the nicotinic receptor antagonist d-TC blocked most of the sEPSC events. Using this system, we found that treatment with therapeutic concentrations of clozapine acutely and chronically suppressed the frequency and amplitude of sEPSCs, without affecting their kinetics. These data suggest that clozapine inhibits both presynaptic release probability and postsynaptic channels.

Cholinergic transmission occurs through similar receptors and mechanisms in both the CNS and PNS, and in the CNS, nicotinic cholinergic involvement in cognitive functioning has been well studied. Although clozapine-induced memory and attentional impairment has been observed in intact experimental animals (Addy & Levin, 2002; Rezvani & Levin, 2004), clozapine in numerous studies improved cognitive function in patients with schizophrenia (McGurk, 1999). Interactions with the cholinergic system have been suggested to play an important role in these cognitive restorative effects of clozapine (Meltzer & McGurk, 1999). Both typical and atypical APDs have strong anticholinergic effects in the frog neuromuscular junction (Boucher & Katz, 1977; Cheng et al., 1981; Nguyen & Miledi, 2002; Sahaf & Publicover, 1986). The effects of these drugs on nicotinic neurotransmission in the CNS are less well known, possibly because the electrophysiological characterization of nicotinic transmission in central neurons is technically challenging (Alkondon et al., 1998). Since all neuronal nAChR have subunit profiles resembling that of ganglionic nicotinic receptors, assessing the effects of APDs on ganglionic nicotinic receptors is of particular interest for documenting the actions of APDs (Daly, 2005). The cholinergic synapse formed between SCG neurons in long-term culture has been widely used to study fast synaptic transmission (Krapivinsky et al., 2006; Luther et al., 2013; Ma & Mochida, 2007; Mochida et al., 1994; Nai et al., 2010). In our study, d-TC, a relatively nonselective antagonist of nicotinic receptors, blocks most of the sEPSCs, suggesting that these synaptic events are mediated mainly by nicotinic receptors. The transmitter of SCG synapses switches from noradrenaline to acetylcholine (Ma & Mochida, 2007) and multiple nicotinic receptor subunits are expressed in cultured SCG neurons (De Koninck & Cooper, 1995). Therefore, it would be of interest to explore whether other nicotinic receptor antagonist and adrenergic receptor antagonists block the remaining sEPSCs. Nonetheless, the SCG preparation is a powerful model for detecting nicotinic effects of drugs, as studied here.

Our data provide evidence on how therapeutic concentrations of clozapine may directly regulate nicotinic transmission at a fast cholinergic synapse. Many reports have suggested that therapeutic plasma clozapine concentration typically ranges between approximately 1 and 2 μM (Broich et al., 1998; Chang et al., 1997). However, some clinical studies have suggested that therapeutic effects of clozapine could require higher plasma concentrations of clozapine than 1 μM in treatment-refractory schizophrenic patients (Hasegawa et al., 1993; Perry et al., 1991). Clozapine concentrations around 10 μM in schizophrenic patients seem to cause toxic adverse effects, such as anticholinergic syndromes, including sinus tachycardia (Chang et al., 1997). Therefore, it is likely that most therapeutic effects, e.g. the improvement of cognition, not the toxic adverse effects, are produced by concentrations at or close to our studies and that the clozapine-induced inhibition of nAChRs we observe might contribute to causing or fully mediate those therapeutic effects.

The reduction of sEPSC frequency and amplitude by both acute and chronic clozapine suggests that clozapine may inhibit both presynaptic acetylcholine release mechanisms and postsynaptic nicotinic receptor responsiveness. Clozapine has been reported to inhibit various nAChR subunit mediated currents in a number of in vitro and in situ preparations (Nguyen & Miledi, 2002; Singhal et al., 2007; Grinevich et al., 2009). Thus, the inhibition of sEPSC amplitude by clozapine in our study is likely a direct effect on postsynaptic receptors. In contrast, the inhibition of sEPSCs frequency suggests an inhibition of presynaptic release and/or postsynaptic receptors. In the CNS, nAChRs are located mainly presynaptically, modulating calcium-dependent terminal release of neurotransmitters including dopamine, norepinephrine, glutamate, GABA, and ACh itself (Wonnacott et al., 2006). nAChRs are nonetheless expressed on dendrites and cell bodies and the suppression of sEPSC frequency may also be due to direct inhibition of postsynaptic nAChRs. Therefore, in our vitro study, the total effect of clozapine on nicotinic transmission is a decrease of ACh release and/or a direct inhibition of nAChRs. Additional studies in the future, e.g. paired-pulse ratio analysis of evoked EPSCs between connected neurons under the dual-patch recording configuration or analysis of postsynaptic currents elicited by focal puffing of exogenous ACh can help elucidate the precise pre- or postsynaptic mechanisms involved in clozapine regulation of cholinergic neurotransmission. Finally, the decreases in sEPSC frequency and amplitude by clozapine might in part be a result of reduced action potential firing. However, it is currently unknown whether clozapine inhibits membrane excitability in SCG neurons, and this possibility needs to be examined in the future.

In vivo studies have shown that clozapine increases ACh release and that this modulation involves indirect mechanisms, through 5-HT_{1A}, 5-HT_{2A}, D₂, α _{1/2} adrenoceptor and muscarinic M₂ receptors (Chung et al., 2004; Ichikawa et al., 2002; Johnson et al., 2005; Shirazi-Southall et al., 2002). The apparent contradiction between in vivo and in vitro studies might not be surprising considering that neural circuits and synaptic connections in the intact brain are far more complicated than those in tissue preparations and that clozapine may simultaneously affect many receptors and molecular targets directly or indirectly. Increased release of ACh might be able to overcome the nicotinic receptor blockade induced by clozapine, although endogenous agonists can rarely reach concentrations needed to overcome exogenous antagonists. Through increased release ACh, clozapine may be an indirect cholinergic agonist as well as a direct-acting antagonist for nAChRs. The overall

consequences of clozapine effects on cholinergic neurotransmission remain to be determined. In behavioral studies, clozapine has been reported to have paradoxical effects, in which clozapine impairs memory and attention in intact experimental animals (Addy & Levin, 2002; Rezvani & Levin, 2004), whereas it tends to improve cognitive function in patients with schizophrenia (McGurk, 1999). Thus, the action of clozapine on cholinergic transmission may depend on the disease status. In patients with schizophrenia, abnormal cortical cholinergic input systems have been implicated (Lewis & Picciotto, 2013) and clozapine may act as a “balancer” or regulator for those abnormal systems. For example, chronic clozapine treatment can normalize the disrupted mesolimbic regulation of prefrontal cholinergic transmission in an animal model of schizophrenia, in which ACh release in prefrontal cortex (PFC) is induced by stimulation of NMDA receptor (Alexander et al., 2009).

In our study, chronic treatment with 2 μ M clozapine significantly decreased the amplitude and frequency of sEPSCs. However, we noticed that, compared to acute administration of the same dose, the effects were in fact more modest. Chronic treatment with 0.5 μ M clozapine had no effects on sEPSCs. This may be due to functional adaptation to the drug or ultrastructural changes in the culture over time, or both. Clozapine treatment in culture increases the density of dendritic spine and up-regulates the number of postsynaptic densities at glutamatergic synapses (Critchlow et al., 2006). In primates, after chronic clozapine exposure, the proportion of nuclei expressing the neuronal marker NeuN increased significantly in subcortical white matter (Halene et al., 2016). Our data show that therapeutic concentrations of clozapine do not affect the neuronal survival rate; however, we cannot exclude potential presynaptic and postsynaptic ultrastructural changes at the nicotinic synapse. The molecular mechanisms underlying the long-term effects of clozapine may involve changes in gene expression triggered by inhibition of neurotransmitter receptors, such as dopamine receptors, which are known molecular targets of clozapine (Seeman and Van Tol, 1994; Gray and Roth 2007). Clozapine is also emerging as an activator of DNA demethylation (Dong et al., 2008; Guidotti and Grayson, 2014) and a regulator of histone H3K4 at certain gene promoters (Shen et al., 2014), thus it can influence chromatin remodeling and gene expression, accounting for potential functional and/or structural changes at SCG synapses. Finally, potential homeostatic plasticity in response to clozapine-induced changes in action potential firing in SCG neurons cannot be ruled out.

Schizophrenia is a complex psychiatric disorder clinically categorized into three main symptom domains: positive (hallucinations and delusions), negative (social withdrawal and apathy) and cognitive deficits. The two primary classes of drugs used in the treatment of schizophrenia are typical (e.g., haloperidol) and atypical (e.g., clozapine) antipsychotics and they differ in both their efficacy at treating the symptom domains of schizophrenia and their side effect profiles. The atypical APDs are often preferable due to their lesser extrapyramidal side effects. Among the atypical APDs, only clozapine has repeatedly demonstrated higher efficacy in treatment-resistant patients and in treating negative symptoms. Abnormalities in the cholinergic system are implicated in cognitive deficits in schizophrenia patients (Lewis & Picciotto, 2013; Olincy & Freedman, 2012) and APDs, especially clozapine, are now recognized to improve cognitive dysfunctions in schizophrenics (Meltzer & McGurk, 1999). The inhibitory modulation on cholinergic transmissions in cultured mammalian SCG

neurons by clozapine may be one effect contributing to cognitive normalization and further study of this action may help advance understanding of the underlying mechanisms.

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Fig. 1.

d-tubocurarine reversibly blocked the majority of sEPSCs in cultured SCG neurons. sEPSCs were recorded in standard external saline at -60 mV. A. Sample recordings under control condition (top), treatment with d-TC (middle) and washout of d-TC (bottom). The nicotinic receptor blocker d-tubocurarine (d-TC, $100 \mu\text{M}$) was acutely applied to the extracellular solution after recording the baseline for five minutes. d-TC reversibly inhibited both the amplitude (B) and frequency (C) of the sEPSCs without affecting the rise time and decay time (D and E). $n = 5$.

Fig. 2.

Acute clozapine treatment concentration-dependently decreased both the amplitude and frequency of sEPSCs.

A and B, Sample traces of sEPSCs in normal bath (top), bath containing 0.5 μ M or 2 μ M (middle), and washout of clozapine (bottom). C, D, E and F, summary acute clozapine effects. Clozapine at 0.5 μ M and 2 μ M significantly decreased the amplitude (C) and frequency (D) of sEPSCs, but did not affect the rise time (E) and decay time (F). The inhibition of amplitude and frequency was reversible. Note that the small outward “blips” in (B) are electric noises.

Fig. 3.

SCG neuron morphology and cell counts under different conditions.

Treatments started at DIV10 and maintained for 4 days. Clozapine was dissolved in DMSO to desired final concentrations. DMSO alone was added in control well. Final concentration of DMSO in medium was 0.1%. Cells were imaged 4 days after treatment in control medium (A), medium containing 0.5 μM clozapine (B), and medium containing 2 μM clozapine (C), respectively. D, Neurons were counted after 4 days of treatment and normalized to control group (normalized neuronal counts, NNC). The numbers in the treatments did not differ from control wells. Pictures were taken on DIV14 with 20X magnification.

Fig. 4.

Chronic clozapine treatment concentration-dependently decreased the amplitude and frequency of sEPSCs.

Recordings were performed after clozapine treatment for 4 days. The culture medium was replaced with standard external saline before recordings. A, Traces were recorded in treatment with 0.1% DMSO (top), 0.5 μ M (middle), or 2 μ M clozapine (bottom). 2 μ M, but

not 0.5 μ M clozapine significantly decreased the amplitude (B), and frequency (C) of sEPSCs, but had no effect on the rise time (D) and decay time (E) of sEPSCs.

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