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RESEARCH PAPER

Allosteric modulation of GluN2C/GluN2D-containing NMDA receptors bidirectionally modulates dopamine release: implication for Parkinson's disease

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BACKGROUND AND PURPOSE

Allosteric modulators of ionotropic receptors and GPCRs might constitute valuable therapeutic tools for intervention in several diseases, including Parkinson's disease (PD). However, the possibility that some of these compounds could alter neurotransmission in health and disease has not been thoroughly examined. Hence, we determined whether CIQ, a positive allosteric modulator of NMDA receptors that contain the GluN2C or GluN2D subunits, modulates dopamine release in the striatum of control mice and of a mouse model of presymptomatic Parkinsonism.

EXPERIMENTAL APPROACH

We used amperometry to measure, in mouse brain slices containing the dorsal striatum, dopamine release evoked by stimulations that mimicked tonic (single pulses) or phasic (trains) activity. We used control mice and mice with a partial, 6-hydroxydopamine-induced, degeneration of dopaminergic neurons in the substantia nigra.

KEY RESULTS

In control mice, CIQ inhibited tonic dopamine release and induced an initial inhibition followed by a long-lasting increase in phasic release. Pirenzepine, a muscarinic receptor antagonist, blocked the depression of release induced by CIQ, but not the long-lasting potentiation. CIQ also increased action potential firing in striatal cholinergic interneurons. In the partially dopamine-depleted striatum, CIQ induced an inhibition followed by a potentiation of both tonic and phasic release, but did not significantly increase the firing of cholinergic interneurons.

CONCLUSIONS AND IMPLICATIONS

CIQ has bidirectional, activity- and ACh-dependent, modulatory effects on dopamine release in the striatum. This study suggests a potentially valuable means to enhance dopamine release in presymptomatic Parkinsonism.

Abbreviations

6-OHDA, 6-hydroxydopamine; aCSF, artificial CSF; CIQ, (3-chlorophenyl)(6,7-dimethoxy-1-[(4-methoxyphenoxy) methyl]-3,4-dihydroisoquinolin-2(1*H*)-yl)methanone; PD, Parkinson's disease; TH, tyrosine hydroxylase

Introduction

In Parkinson's disease (PD), degeneration of dopaminergic neurons in the substantia nigra pars compacta leads to an imbalance in several other neurotransmitter systems in the basal ganglia, including glutamatergic neurotransmission (Loftis and Janowsky, 2003; Gogas, 2006). Interactions between glutamate and dopamine in the striatum, which play important roles in motor control, are thus altered in PD. Broad-spectrum antagonists of the *N*-methyl-D-aspartate (NMDA) type of glutamate receptors alleviate some of the motor symptoms of PD but these beneficial effects are accompanied by unwanted side effects. Because the subunit composition of NMDA receptors determines physiological and pathophysiological processes involving these receptors, subunit-specific NMDA receptor antagonists have been investigated as possible therapeutic agents for treatment of PD with reduced propensity to elicit side effects (Hallett and Standaert, 2004). NMDA receptors are heterotetrameric assemblies of two GluN1 subunits and other GluN2 (A–D) and GluN3 (A, B) subunits (Alexander *et al.*, 2013b; Paoletti *et al.*, 2013). GluN2B is an attractive drug target for therapeutic intervention in PD; unfortunately, clinical trials with GluN2B-selective antagonists failed to provide clear benefit in PD patients (Kalia *et al.*, 2013). Novel therapeutic strategies for treatment of PD targeting the GluN2 subunits that compose NMDA receptors in the basal ganglia are warranted. The GluN2D subunit contributes to functional NMDA receptors in midbrain dopaminergic neurons (Brothwell *et al.*, 2008) and might therefore play a role in the modulation of dopamine release in the striatum. In the striatum, GluN2D is expressed in interneurons, particularly large cholinergic interneurons (Landwehrmeyer *et al.*, 1995; Bloomfield *et al.*, 2007). These interneurons play key roles in the physiology of the striatum and have been implicated in the pathophysiology of PD (Pisani *et al.*, 2007).

Positive and negative allosteric modulators of ionotropic receptors and GPCRs offer the possibility to enhance or decrease the function of the targeted receptors when stimulated by the endogenous neurotransmitter. Such compounds might constitute valuable therapeutic tools for intervention in several diseases, including PD. CIQ is a positive allosteric modulator of NMDA receptors that contain the GluN2C or GluN2D subunits. This recently developed compound was shown to potentiate NMDA responses in neurons of the subthalamic nucleus, which express GluN2D, and to have no effect on NMDA responses in hippocampal pyramidal neurons, which express mostly GluN2A and GluN2B (Mullasseril *et al.*, 2010; Ogden and Traynelis, 2011). CIQ was recently shown to enhance fear acquisition and increase fear extinction when infused in the amygdala (Ogden *et al.*, 2014). The possible effects of CIQ on neurotransmission in health and disease have not been investigated yet. The aim of our study was therefore to examine whether CIQ affects dopamine release and the activity of cholinergic interneurons in the striatum of control mice. We also investigated whether the effects of CIQ were altered in mice with a partial damage of nigrostriatal dopaminergic neurons, a model of presymptomatic PD. Our results demonstrate that CIQ bidirectionally modulates dopamine release in the striatum of control and partially dopamine-depleted mice. They also suggest the involvement of striatal cholinergic interneurons in the inhibitory effect of CIQ, which is reduced in the partially dopamine-depleted striatum.

Methods

The nomenclature of NMDA receptor subunits conforms to the *British Journal of Pharmacology's Concise Guide to PHARMACOLOGY* (Alexander *et al.*, 2013a,b).

Animals and brain slice preparation

Experiments were approved by our local ethical committee (Stockholms norra djurförsöksetiska nämnd) and were performed as described previously (Schotanus and Chergui, 2008; Zhang *et al.*, 2008; Chergui, 2011). All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010: McGrath *et al.*, 2010). All efforts were made to minimize animal suffering. We used 32 male C57BL/6 mice 4–9 weeks-old (Harlan Laboratories, Boxmeer, the Netherlands). Mice were maintained on a 12:12 h light/dark cycle and had free access to food and water. Control mice did not undergo surgery before the experiments. Another group of mice underwent unilateral stereotaxic injection of the toxin 6-hydroxydopamine (6-OHDA) in the substantia nigra pars compacta to produce partial degeneration of dopaminergic neurons and partial lesion of the dopaminergic innervation of the striatum. These mice were anaesthetized with an i.p. injection of 80 $mg \cdot kg^{-1}$ ketamine and 5 $mg \cdot kg^{-1}$ xylazine. The depth of the anaesthesia in the mice was assessed by the tail/paw pinching test. Mice were sufficiently anaesthetized when they did not respond to these stimuli. Mice were placed in a stereotaxic frame and injected, over 2 min, with 1.5 µg 6-OHDA in 0.01% ascorbate into the substantia nigra pars compacta of the right hemisphere. The coordinates for injection were AP: –3 mm; ML: –1.1 mm; and DV: –4.5 mm relative to bregma and the dural surface (Paxinos and Franklin, 2001). The mice underwent cervical dislocation followed by decapitation (for lesioned mice, this was carried out 1–3 weeks after the surgery). Their brains were rapidly removed and brain slices (coronal and sagittal, 400 µm thick) containing the striatum and the overlying cortex were prepared with a microslicer (VT 1000S; Leica Microsystem, Heppenheim, Germany). Slices were incubated, for at least 1 h, at 32°C in oxygenated (95% O_2 + 5% CO_2) artificial CSF (aCSF) containing (in mM): 126 NaCl, 2.5 KCl, 1.2 NaH_2PO4, 1.3 $MgCl_2$, 2.4 $CaCl_2$, 10 glucose and 26 $NaHCO_3$, pH 7.4. Slices were transferred to a recording chamber (Warner Instruments, Hamden, CT, USA; recording chamber from Scientifica Ltd., Uckfield, UK) mounted on an upright microscope (Olympus, Solna, Sweden and Scientifica Ltd.) and were continuously perfused with oxygenated aCSF at 28°C.

Amperometry and electrophysiology in brain slices

Amperometric detection of dopamine release was performed with carbon fibre electrodes (10 µm diameter; World Precision Instruments Europe, Aston, Stevenage, UK), which had an active part of 100 µm that was positioned within the striatum in the brain slice. A constant voltage of +500 mV was

applied to the carbon fibre via an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA) and currents were recorded with the same amplifier. A stimulating electrode (patch electrode filled with aCSF) was placed on the slice surface, in the vicinity of the carbon fibre electrode. Stimulations consisted of either single pulses or trains of 4 pulses at 100 Hz (0.1 ms, 8–14 μA), applied every min or every 2 min, which evoked a response corresponding to oxidation of dopamine at the surface of the electrode.

Cell-attached recordings of cholinergic interneurons in the dorsal striatum were made with the help of infrared-differential interference contrast video microscopy. Cholinergic interneurons were identified by their morphological and electrophysiological properties, which include a large soma, spontaneous firing, pronounced long-lasting spike afterhyperpolarization, resting membrane potential around –60 mV (Kawaguchi, 1993). Patch electrodes were filled with a solution containing, in mM: 120 D-gluconic acid, 20 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 EGTA, 2 MgATP, 0.3 Na_3GTP, pH adjusted to 7.3 with KOH. Whole-cell membrane currents and potentials were recorded with a MultiClamp 700B (Axon Instruments), acquired at 10 kHz and filtered at 2 kHz.

Western blotting

Western blots were performed to confirm loss of tyrosine hydroxylase (TH) following 6-OHDA lesioning in the slices that were used for electrophysiological experiments. The slices were frozen and stored at –20°C until processed. The samples were sonicated in 1% SDS and boiled for 10 min. Protein concentration was determined in each sample with a bicinchoninic acid protein assay (BCA-kit, Pierce, Rockford, IL, USA). Equal amounts of protein (30 μg) were re-suspended in sample buffer and separated by SDS-PAGE using a 10% running gel and transferred to an Immobilon-P (polyvinylidene difluoride) transfer membrane (Sigma-Aldrich). The membranes were incubated for 1 h at room temperature with 5% (w v^{-1}) dry milk in TBS–Tween20. Immunoblotting was carried out with antibodies against TH (Millipore, Billerica, MA, USA) and β-actin (Sigma-Aldrich) in 5% dry milk dissolved in TBS–Tween 20. The membranes were washed three times with TBS–Tween20 and incubated with secondary HRP-linked anti-rabbit IgG (H + L) (Thermo Scientific, Göteborg, Sweden; 1:6000 dilution) for 1 h at room temperature. At the end of the incubation, membranes were washed six times with TBS–Tween 20 and immunoreactive bands were detected by enhanced chemiluminescence (PerkinElmer, Waltham, MA, USA). The autoradiograms were scanned and quantified with NIH Image 1.63 software (NIH, Bethesda, MD, USA). The levels of protein were normalized for the value of β-actin. We found that the levels of β-actin were similar in the intact and in the dopamine-depleted striatum (99.6 ± 3.6% of intact), and therefore, we have not determined the levels of other housekeeping genes. Data were analysed with the Mann–Whitney test to evaluate statistical differences between intact and lesioned striatum.

Data acquisition and analysis of amperometric and electrophysiological measurements

Data were acquired and analysed with the pClamp 9 or pClamp 10 software (Axon Instruments). Numerical values are shown as means with SEM, with *n* indicating the number of slices or neurons tested. For dopamine release, data are expressed as % of the baseline response measured for each slice during the 5–10 min preceding start of perfusion with CIQ. Statistical significance of the results was assessed by using Student's *t*-test for paired observations (comparisons with baseline within single groups) or one-way ANOVA multiple comparison test followed by Dunnett's test (comparisons between different groups).

Chemicals and drugs

Chemicals and drugs were purchased from Sigma-Aldrich (Stockholm, Sweden), Tocris Bioscience (Bristol, UK) and AbcamBiochemicals (Cambridge, UK). All compounds were prepared in stock solutions, diluted in aCSF to the desired final concentration and applied in the perfusion solution. The following compounds were used (final concentrations in μM): CIQ [(3-chlorophenyl)(6,7-dimethoxy-1-[(4-methoxyphenoxy)methyl]-3,4-dihydroisoquinolin-2(1*H*)-yl)methanone (20)] racemate, mecamylamine (5), pirenzepine dihydrochloride (1) and UBP141 (6). CIQ was dissolved in DMSO to obtain a 100 mM stock solution, which was aliquoted and stored at –20°C. On the day of the experiment, single aliquots were thawed and 20 μM solutions were made in aCSF. Stirring and oxygenation of the solutions containing CIQ were required to avoid precipitation of the compound. The effects of CIQ on dopamine release were examined within around 10 min after these solutions were prepared. We used the competitive GluN2C/GluN2D-preferring antagonist UBP141, which displays 5- to 10-fold selectivity for GluN2C/GluN2D-containing NMDA receptors over GluN2A/GluN2B-containing NMDA receptors (Feng *et al.*, 2004; Costa *et al.*, 2009). The concentration of this compound used in our study was previously shown to inhibit synaptic and extrasynaptic NMDA receptor-mediated currents in hippocampal and midbrain slices with minimal effect on receptors containing GluN2A or GluN2B (Brothwell *et al.*, 2008; Harney *et al.*, 2008; Costa *et al.*, 2009; Harney and Anwyl, 2012). We also found that, in the striatum, 6 μM UBP141 decreased NMDA receptor-mediated responses in cholinergic interneurons, which express GluN2D, but not in projection neurons, which do not express GluN2D in the adult striatum (unpublished observations and see Feng *et al.*, 2014). Because GluN2C is absent from the striatum (Bloomfield *et al.*, 2007), UBP141 probably antagonizes the action of CIQ on GluN2D-containing NMDA receptors. Pirenzepine is a muscarinic receptor antagonist (Caulfield and Birdsall, 1998) with some preference for M_1 receptors (Doods *et al.*, 1994) and partial affinity for M_4 muscarinic receptors. Previous studies have used pirenzepine in concentrations between 1 and 10 μM to examine the role of muscarinic M_1 receptors in glutamatergic synaptic transmission and plasticity in striatal brain slices (Wang *et al.*, 2006; Tozzi *et al.*, 2011). We therefore selected a 1 μM concentration to investigate the role of muscarinic receptors in the effects of CIQ. As shown in previous studies (Rice and Cragg, 2004; Zhang and Sulzer, 2004), the nicotinic receptor antagonist mecamylamine applied alone in the perfusion solution depressed dopamine release evoked in the striatum (to 37.8% ± 9.8 of baseline, n = 10 slices), demonstrating a tonic activation of these receptors in the slice.

Results

Effects of CIQ in the striatum of control mice

We first evaluated the effect of CIQ on dopamine release, measured with amperometry coupled to carbon fibre electrodes, in corticostriatal brain slices from control mice. We applied CIQ in the perfusion solution at a concentration (20 μM), which was demonstrated to increase NMDA-activated currents in neurons of the subthalamic nucleus in brain slices (Mullasseril *et al.*, 2010). We found that, in the striatum, CIQ (20 μM) depressed dopamine release evoked by single stimulation pulses, which mimic tonic activity in dopaminergic neurons (Chergui *et al.*, 1994) ($n = 9$; Figure 1). We then examined whether GluN2D-containing NMDA receptors contributed to the effect of CIQ in our experimental conditions. CIQ did not affect dopamine release in the presence of UBP141 (6 μM), a GluN2D-containing NMDA receptor antagonist ($n = 6$; Figure 1C). These results show that the depressant action of CIQ is mediated by NMDA receptors that contain GluN2D.

We then investigated the locus of action of CIQ and we examined the involvement of striatal cholinergic interneurons. These interneurons express GluN2D (Landwehrmeyer *et al.*, 1995; Bloomfield *et al.*, 2007), and ACh acting on muscarinic receptors exerts a powerful inhibitory effect on dopamine release (Zhang and Sulzer, 2012). CIQ-induced depression of dopamine release evoked by single stimulation pulses was blocked in the presence of pirenzepine (1 μM), a muscarinic receptor antagonist ($n = 8$; Figure 1C). These results suggest that ACh, released following an increased activity in cholinergic interneurons, could contribute to CIQ-induced inhibition of dopamine release. To test this hypothesis, we examined the effect of CIQ on action potential firing in cholinergic interneurons recorded in the cell-attached configuration. CIQ increased the firing rate of spontaneously active cholinergic interneurons in the striatum of control mice ($n = 6$; Figure 2). This increased activity could therefore contribute to the depressant action of CIQ on dopamine release.

Earlier studies demonstrated that ACh controls dopamine release in the striatum in an activity-dependent manner, thus enhancing the phasic versus tonic contrast (Zhang and Sulzer, 2012). We investigated the activity dependence of the modulatory effects of CIQ and found that this compound induced an initial short lasting inhibition of dopamine release evoked by stimulation trains (four stimulation pulses at 100 Hz) that mimic phasic activity ($n = 7$; Figure 3). This short-lasting depression of release was blocked in the presence of pirenzepine ($n = 6$; Figure 3C). Inhibition was however followed by a long-lasting potentiation of release (Figure 3), which was not blocked by pirenzepine (Figure 3C). We then examined the possibility that CIQ could mediate its effects via nicotinic ACh receptors. Indeed, in a recent study CIQ was shown to have modest inhibitory effect at nicotinic ACh receptors (Ogden *et al.*, 2014). We found that the inhibitory and potentiating effects of CIQ on phasic dopamine release were not significantly different in the presence of the nicotinic receptor antagonist mecamylamine (5 μM, $n = 11$) as compared with control slices (Figure 3C). These results show that the actions of CIQ on dopamine release are not mediated through nicotinic receptors.

Effects of CIQ in the partially dopamine-depleted striatum

We then examined the effect of CIQ on dopamine release in a mouse model of presymptomatic PD, in which a number of dopaminergic axon terminals are still able to release dopamine despite a significant reduction in the number of dopaminergic neurons. The toxin 6-OHDA was injected in the substantia nigra pars compacta of a group of mice to produce partial damage of the dopaminergic neurons. In the partially lesioned striatum, evoked-dopamine release was detectable although the peak current amplitude was much smaller than in control slices (Figure 4B and D). CIQ induced an initial depression of release evoked by single stimulation pulses and by trains, which was not significantly different in magnitude as compared with inhibition observed in control mice ($P > 0.05$). However, this depression was short lasting because it

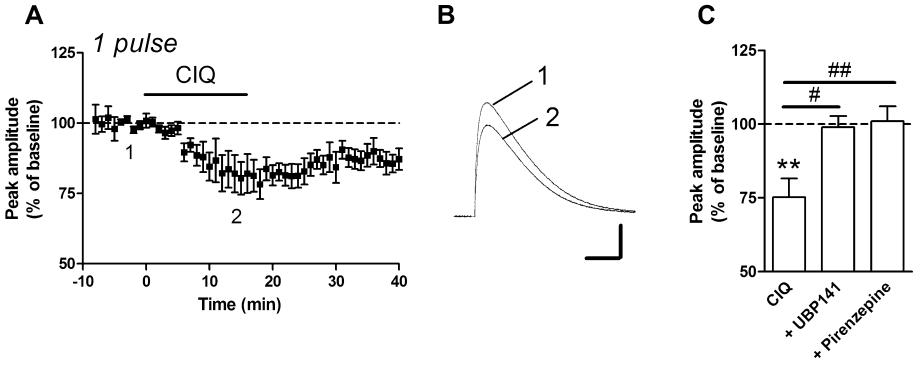

Figure 1

CIQ inhibits tonic dopamine release in the striatum of control mice. (A) Time course of the effect of CIQ (20 μM) on dopamine release evoked by single stimulation pulses ($n = 9$). (B) Representative amperometric traces from one slice, at the time points indicated in (A), that is, before (1) and during (2) perfusion with CIQ. Scale bars: 30 pA and 50 ms. (C) Average magnitude of the effect of CIQ on dopamine release in control slices ($n = 9$), in slices perfused with the GluN2D-containing NMDA receptor antagonist UBP141 (6 μM; $n = 6$), and with the muscarinic receptor antagonist pirenzepine (1 μM; $n = 8$). $^{**}P < 0.01$, compared with baseline (Student's *t*-test); $\#P < 0.05$, $\#\#P < 0.01$ compared with control slices (ANOVA).

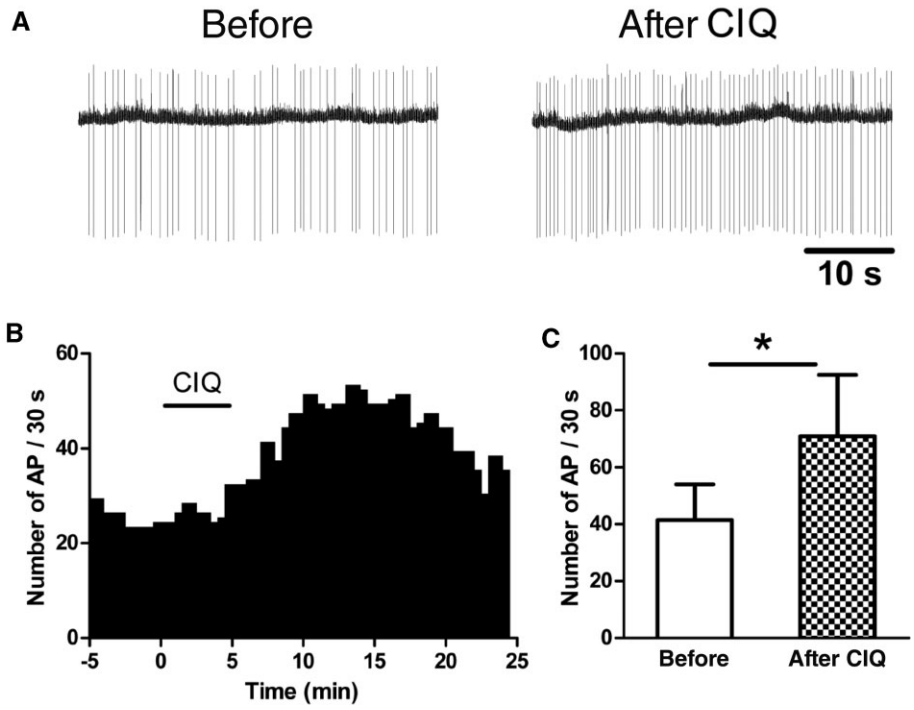

Figure 2

CIQ increases the firing of cholinergic interneurons in the striatum of control mice. (A) Firing in a cholinergic interneuron, measured in somatic cell-attached mode, before and after perfusion with CIQ (20 μM). (B) Time histogram of the firing in the neuron presented in (A). CIQ was applied in the perfusion solution at the time indicated by the black bar. (C) Average firing in cholinergic interneurons ($n = 6$), before and after bath application of CIQ. *$P < 0.05$ compared with baseline firing in the same neurons (Student's *t*-test).

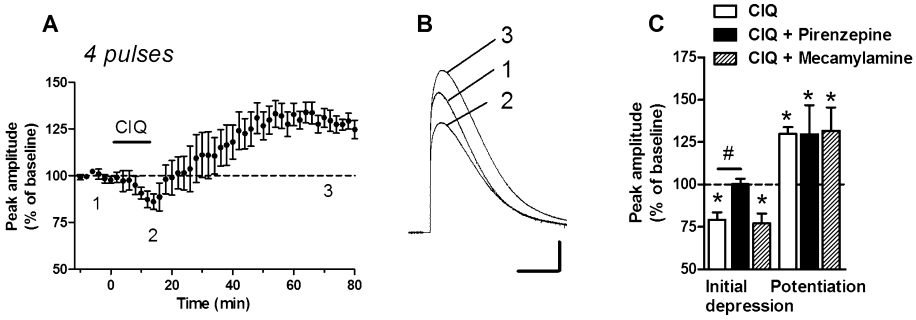

Figure 3

CIQ bidirectionally modulates phasic dopamine release in the striatum of control mice. (A) Time course of the effect of CIQ (20 μM) on dopamine release evoked by stimulation trains (4 pulses at 100 Hz; $n = 7$). (B) Representative amperometric traces from one slice, at the time points indicated in (A). Scale bars: 30 pA and 50 ms. (C) Average magnitude of CIQ-induced initial depression and delayed potentiation in control slices ($n = 7$), in slices perfused with pirenzepine ($n = 6$) and in slices perfused with mecamylamine ($n = 11$). *$P < 0.05$, compared with baseline (Student's *t*-test); #$P < 0.05$, compared with control slices (ANOVA).

was followed by an increased release evoked by both single stimulation pulses and trains, in 8 out of 11 slices examined (Figure 4A–D).

Western blot analyses confirmed that the protein levels of TH, the rate-limiting enzyme in the synthesis of dopamine, were reduced to 48% of the amounts in the intact striatum from the same mice (Figure 4E). In addition, we found that CIQ did not significantly increase the firing of cholinergic interneurons in partially lesioned striatum ($n = 5$; Figure 4F).

Discussion

The expression of the different GluN2 subunits that compose functional NMDA receptors is developmentally regulated. Low levels of GluN2D are detected in the striatum of 7-day-old rats but this subunit is undetectable in 49-day-old rats (Dunah *et al.*, 1996). In line with these observations, functional NMDA receptors in medium spiny projection neurons were shown to contain GluN2C/2D in immature and young

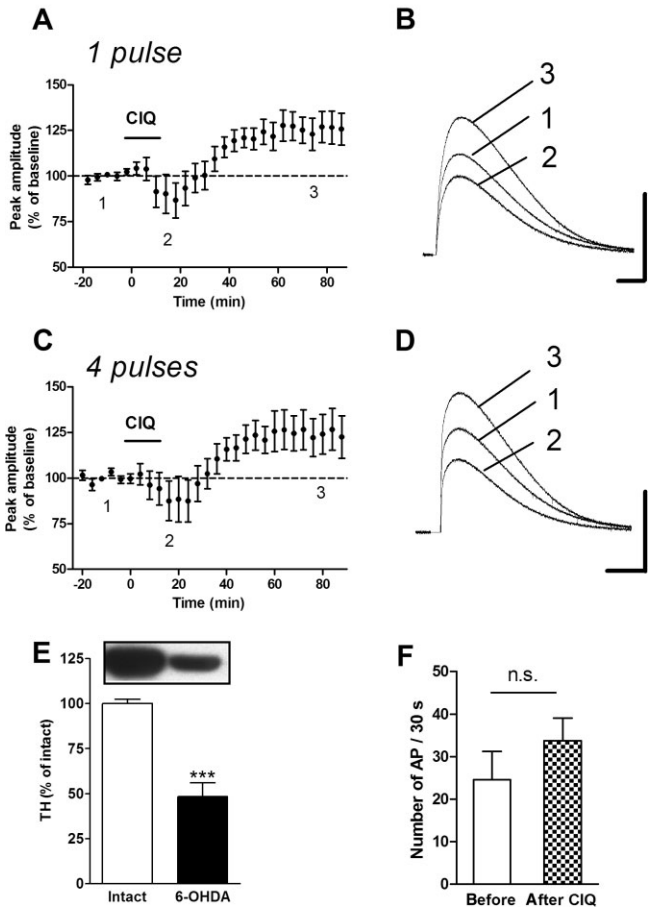

Figure 4

CIQ potentiates tonic and phasic dopamine release in the partially lesioned striatum. (A–D) Effect of CIQ (20 μM) on dopamine release evoked by single pulses (A, B; $n = 11$) and by trains (C, D; $n = 11$) in the striatum of mice with a partial 6-OHDA-induced loss of dopaminergic neurons. Scale bars: 30 pA and 50 ms. (E) Protein levels of TH in intact and 6-OHDA-lesioned striatum, measured in the slices used in (A–D). ***$P < 0.001$, compared with intact striatum (Mann–Whitney test). (F) Average firing in cholinergic interneurons before and after bath application of CIQ (20 μM) in the partially dopamine-depleted striatum ($n = 5$, n.s., not statistically significant, Student's *t*-test).

(7–20 days old) mice but not in older rodents (Logan *et al.*, 2007; Tong and Gibb, 2008; Dehorter *et al.*, 2011). In older mice, functional NMDA receptors in the striatal complex contain mostly GluN2A and GluN2B (Chergui, 2011). GluN2D is, however, expressed in striatal interneurons, particularly large cholinergic interneurons (Landwehrmeyer *et al.*, 1995; Standaert *et al.*, 1996; Bloomfield *et al.*, 2007). Dopaminergic neurons in the substantia nigra pars compacta provide a dense innervation of the striatum (Gerfen, 1992). These neurons express functional NMDA receptors that contain GluN2B and GluN2D (Wenzel *et al.*, 1996; Jones and Gibb, 2005; Brothwell *et al.*, 2008; Suárez *et al.*, 2010).

In the present study, we show that CIQ, a positive allosteric modulator of NMDA receptors that contain GluN2C or GluN2D, modulates dopamine release in brain slices from the intact and partially dopamine-depleted striatum in an activity- and ACh-dependent manner. These effects are most likely mediated through GluN2D-containing NMDA receptors. Although CIQ is not an agonist, this compound modulates evoked-dopamine release in the striatum and increases the firing of cholinergic interneurons. An endogenous concentration of glutamate in the slice, as well as glutamate released upon stimulation of the slice, might activate GluN2D-containing NMDA receptors.

Nevertheless, our results demonstrate that CIQ depresses both tonic and phasic release of dopamine through a mechanism that probably involves an increased firing in cholinergic interneurons and subsequent release of ACh. On the contrary, CIQ increases phasic release of dopamine through a mechanism that does not require ACh muscarinic receptors and is probably independent of the activity of cholinergic interneurons. Direct presynaptic activation of GluN2D-containing NMDA receptors located on dopaminergic terminals could contribute to the potentiating action of CIQ. Thus, the bidirectional, activity-dependent, effect of CIQ on dopamine release reveals a functional role for GluN2D-containing NMDA receptors in cholinergic interneurons and suggests a possible role for these receptors localized to dopaminergic axon terminals.

In the partially dopamine-depleted striatum, CIQ does not significantly increase the firing of cholinergic interneurons. Because glutamatergic neurotransmission is not reduced, but rather increased, in animal models of PD (Bagetta *et al.*, 2010), our observation suggests that the functions of GluN2D-containing NMDA receptors expressed by cholinergic interneurons are reduced in the 6-OHDA mouse model of presymptomatic PD. A consequence of this change might include an impaired inhibitory control, by GluN2D-containing NMDA receptors, of dopamine release during tonic activity in dopaminergic neurons. Indeed, the depressant action of CIQ on dopamine release evoked by single stimulation pulses is shorter in the partially dopamine-depleted striatum than in the striatum of control mice. This impaired inhibitory control on tonic dopamine release might arise from a reduced release of ACh evoked by CIQ. Interestingly, we found that CIQ induces a long-lasting increase in dopamine release evoked by single stimulation pulses in the partially dopamine-depleted striatum. Taken together with the blockade of CIQ-induced inhibition of dopamine release by a GluN2D antagonist and the lack of effect of a nicotinic receptor antagonist on CIQ-induced actions, these results further demonstrate that CIQ does not affect dopamine release through nicotinic receptors in our experimental conditions. This conclusion is also supported by the observation that the action of nicotinic receptor antagonists on dopamine release is similar in partially lesioned and intact striatum (Perez *et al.*, 2010). Because the symptoms of PD are likely to be related to altered neuronal activity and circuitry in the basal ganglia (Blandini *et al.*, 2000; Bergman and Deuschl, 2002), additional studies are required to determine the effects of CIQ on dopamine release in a more intact preparation than the brain slice, that preserves all connections between the different components of the basal ganglia.

In conclusion, our results demonstrate a dual effect of CIQ in the striatum and thus a bidirectional regulation of dopamine release by GluN2D-containing NMDA receptors. In

control mice, the depressant action of CIQ is dependent upon cholinergic interneurons, whereas CIQ potentiates phasic release independently of cholinergic interneurons. In the partially dopamine-depleted striatum, a potentiating effect of CIQ on both tonic and phasic release is predominant. These results suggest that neuronal adaptations that occur following partial loss of dopaminergic innervation of the striatum include an altered function of GluN2D-containing NMDA receptors, as recently suggested in the total dopamine-depleted striatum (Feng *et al.*, 2014; Zhang *et al.*, 2014). In addition, this study identifies a novel way to enhance dopamine release from residual terminals in presymptomatic Parkinsonism and proposes GluN2D as a potential drug target for therapeutic intervention in PD.

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Author contributions

X. Z. and Z-J. F. performed the experiments and analysed the data. K. C. directed the study and wrote the manuscript. All authors approved the final version of the manuscript.

Conflict of interest

None.

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