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Mefloquine Enhances Nigral γ-Aminobutyric Acid Release via Inhibition of Cholinesterase

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ABSTRACT

Mefloquine, a widely used antimalarial drug, has many neuropsychiatric effects. Although the mechanisms underlying these side effects remain unclear, recent studies show that mefloquine enhances spontaneous transmitter release and inhibits cholinesterases. In this study, we examined the effect of mefloquine on GABA receptor-mediated, spontaneous inhibitory postsynaptic currents (sIPSCs) of dopaminergic neurons, mechanically dissociated from the substantia nigra pars compacta of rats aged 6 to 17 postnatal days. Mefloquine (0.1–10 μM) robustly and reversibly increased the frequency of sIPSCs with an EC_{50} of 1.3 μM. Mefloquine also enhanced the frequency of miniature inhibitory postsynaptic currents in the presence of tetrodotoxin but without changing their mean amplitude. This suggests that mefloquine acts presynaptically to increase GABA release. Mefloquine-induced enhancement of sIPSCs was significantly attenuated in medium containing low Ca^{2+} (0.5 mM) or following pretreatment with 1,2-bis (2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid tetrakis(acetoxymethyl ester (30 μM), a membrane-permeable Ca^{2+} chelator. In contrast, 100 μM Cd^{2+} did not alter the action of mefloquine. This suggests that mefloquine-induced facilitation of GABA release depends on extracellular and intraterminal Ca^{2+} but not on voltage-gated Ca^{2+} channels. Mefloquine-induced enhancement of sIPSCs was significantly attenuated in the presence of the anticholinesterase agent physostigmine or blockers of non-α7 nicotinic acetylcholine receptors. Taken together, these data suggest that mefloquine enhances GABA release through its inhibition of cholinesterase. This allows accumulation of endogenously released acetylcholine, which activates neuronal nicotinic receptors on GABAergic nerve terminals. The resultant increase of Ca^{2+} entry into these terminals enhances vesicular release of GABA. This action may contribute to the neurobehavioral effects of mefloquine.

Mefloquine is a widely used antimalarial drug because of its effectiveness against chloroquine-resistant plasmodia. It is well known that this benefit of mefloquine is offset by many adverse side effects on both the central and peripheral nervous systems (Fuller et al., 2002; Wooltorton, 2002; Falchook et al., 2003; Kukoyi and Carney, 2003; Meier et al., 2004). The most notable adverse effects are neuropsychiatric disturbances of anxiety, confusion, dizziness, and dysphoria. The cellular basis of these effects is not understood.

Synaptic transmission is of great importance in the interplay between cells of the nervous system. Recently, several studies reported that mefloquine potently altered synaptic transmission in rodent central nervous system and peripheral synapses. Specifically, mefloquine robustly enhanced the frequency of spontaneous excitatory postsynaptic potentials in rat hippocampal slices (Cruikshank et al., 2004). Likewise, mefloquine significantly increased the frequency as well as decay time, of miniature end-plate potentials (mEPPS) at the mouse neuromuscular junction (McArdle et al., 2005, 2006). Because the intracellular Ca^{2+} buffer 1,2-bis (2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid tetrakis(acetoxymethyl ester (BAPTA-AM) prevented the effect of mefloquine on mEPP frequency, it was suggested that mefloquine alters storage of Ca^{2+} within motor nerve endings. On the other hand, the prolongation of mEPP decay time appeared to depend on the anticholinesterase action of mefloquine (Lim

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ABBREVIATIONS: mEPPS, miniature endplate potentials; BAPTA-AM, 1,2-bis (2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid tetrakis(acetoxymethyl ester; DA, dopaminergic; SNc, substantia nigra pars compacta; APV, DL-2-amino-5-phosphono-valeric acid; DNQX, 6,7-dinitroquinoxaline-2, 3-dione; TTX, tetrodotoxin; QP, quinpirole hydrochloride; MEC, mecamylamine hydrochloride; DHβE, dihydro-β-erythroidine hydrobromide; α-BgTX, α-bungarotoxin; IPSC, inhibitory postsynaptic current; sIPSC, spontaneous IPSC; K-S, Kolmogorov-Smirnov; mIPSC, miniature IPSC; VGCC, voltage-gated calcium channel; PHY, physostigmine; nAChR, nicotinic acetylcholine receptor; AChE, cholinesterase.

and Go, 1985). The relevance of these findings to the neuropsychiatric effects of mefloquine remains unclear.

Central dopaminergic (DA) neurons regulating cognitive and motor processes are located in the ventral mesencephalon, including the substantia nigra and ventral tegmental area. The substantia nigra pars compacta (SNc) possesses a dense area of DA neurons, receiving GABAergic inhibition primarily from neurons in substantia nigra pars reticulate, pallidum, striatum, and nucleus accumbens (Giustizieri et al., 2005). The GABAergic inputs control the excitability of DA neurons (Tepper et al., 1998). The fact that SNc DA neurons can be isolated along with attached GABAergic terminal boutons (Akaike and Moorhouse, 2003; Ye et al., 2004) presents an opportunity to evaluate the effect of mefloquine on spontaneous GABA release in some detail. The object of this study was to test the hypothesis that mefloquine increases spontaneous GABA release via an interaction with intracellular Ca^{2+} storage and inhibition of cholinesterase.

Materials and Methods

Slice Preparation and Mechanical Dissociation. The care and use of animals and the experimental protocol were approved by the Institutional Animal Care and Use Committee of the University of Medicine and Dentistry of New Jersey. The midbrain slices were prepared as described previously (Ye et al., 2004; Zhou et al., 2006). In brief, rats, aged 6 to 17 postnatal days, were decapitated, and the brain was quickly excised and coronally sliced (300 μm) with a VF-100 Slicer (Precisionary Instruments, Greenville, NC). This was done in ice-cold artificial cerebrospinal fluid saturated with 95% O_2-5% CO_2 (carbogen) containing 126 mM NaCl, 1.6 mM KCl, 1.25 mM NaH_2PO_4, 1.5 mM $MgCl_2$, 2 mM $CaCl_2$, 25 mM $NaHCO_3$, and 10 mM glucose. Midbrain slices were then kept in carbogen-saturated artificial cerebrospinal fluid at room temperature (22–24°C) for at least 1 h before use.

Neurons, with functional terminals, were obtained by mechanical dissociation as described previously (Akaike and Moorhouse, 2003; Ye et al., 2004; Zhou et al., 2006). In brief, slices were transferred to a 35-mm culture dish (Falcon, Rutherford, NJ) filled with a standard external solution containing 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose (320 mOsm, pH set to 7.3 with Tris base). The region of SNc was identified with an inverted microscope (Nikon, Tokyo, Japan). A heavily fire-polished glass pipette with a 50-μm tip in diameter was fixed on a homemade device. The pipette then was positioned by a manipulator to touch slightly the surface of the SNc region. The neurons in the surface of the tissue were dissociated by horizontal vibration at a frequency of 15 to 20 Hz, with a range from 0.1 to 0.3 mm, for 2 to 5 min. The slice was then removed. Within 20 min, the isolated neurons adhered to the bottom of the dish for electrophysiological recording. These mechanically dissociated neurons differed from those neurons dissociated with enzyme. Whereas the latter lost most, if not all, of the nerve terminals during the dissociation process, the former often preserved some functional nerve terminals (Akaike and Moorhouse, 2003; Ye et al., 2004; Zhou et al., 2006).

Electrophysiological Recording. Whole-cell and loose-patch cell attached configurations were used to record electrical activity with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) via a Digidata 1322A analog-to-digital converter (Axon Instruments) and pClamp 9.2 software (Axon Instruments). Data were filtered at 1 kHz and sampled at 5 kHz. The junction potential between the pipette and the bath solutions was nullified just before the giga-seal was formed.

The patch electrodes had a resistance of 3 to 5 MΩ when filled with pipette solution containing 140 mM CsCl, 2 mM $MgCl_2$, 4 mM EGTA, 0.4 mM $CaCl_2$, 10 mM HEPES, 2 mM Mg-ATP, and 0.1 mM GTP. The pH was adjusted to 7.2 with Tris base, and the osmolarity was adjusted to 280 to 300 mOsm with sucrose. Electrophysiological recordings were performed at room temperature (22–24°C).

Chemicals and Applications. Most of the chemicals, including bicuculline, DL-2-amino-5-phosphono-valeric acid (APV), 6,7-dinitroquinoxaline-2,3-dione (DNQX), tetrodotoxin (TTX), (−)-quinpirole hydrochloride (QP), BAPTA-AM, mecamylamine hydrochloride (MEC), dihydro-β-erythroidine hydrobromide (DHβE), α-bungarotoxin (α-BgTX), and physostigmine were purchased from Sigma-Aldrich (St. Louis, MO). All solutions were prepared on the day of the experiment. Mefloquine was kindly provided by Drs. Eva-Maria Gutknecht and Pierre Weber (F. Hoffman-La Roche, Basel, Switzerland). A stock solution (20 mg/ml) of the racemic salt was prepared in dimethyl sulfoxide (Sigma-Aldrich). Dilution of this stock solution into physiological solutions produced the concentrations of mefloquine studied. Chemicals were applied to dissociated neurons with a Y-tube. This exchanged the external solution surrounding the neurons within 40 ms (Zhou et al., 2006).

Data Analyses. Spontaneous inhibitory postsynaptic currents (sIPSCs) were analyzed with Clampfit 9.2 software (Molecular Devices Corporation, Sunnyvale, CA) as described previously (Zhou et al., 2006). In brief, the sIPSCs were screened automatically using a template with an amplitude threshold of 5 pA. These were visually accepted or rejected based upon rise and decay times. More than 95% of the sIPSCs, which were visually accepted, were screened using a suitable template. The amplitudes and interevent intervals of sIPSCs in different conditions were also obtained. Their cumulative probability distributions were constructed using Clampfit 9.2. Following this, a Kolmogorov-Smirnov (K-S) test was used for evaluating the significance of drug effects. EC_{50} was obtained with a logistic equation: $y = y_0 + (ax^b)/(c^b + x^b)$, where y is the drug-elicited percentage change of sIPSC frequency in the presence of concentration x of mefloquine compared with control; a is the difference between maximal effect and minimal effect; and y_0, b, and c denote the minimal effect, Hill coefficients, and half-effective concentration (EC_{50}), respectively. Differences in amplitude and frequency were tested by Student's paired two-tailed t test using their normalized values, unless indicated otherwise. Numerical values are presented as the mean ± S.E.M. Values of $p < 0.05$ were considered significant.

Results

Identification of DA Neurons. DA neurons in SNc were identified on the basis of their well-established pharmacological and electrophysiological properties (Lacey et al., 1989; Zhou et al., 2006). Figure 1A shows sample traces of spontaneous discharges of a DA neuron recorded in the cell-attached mode. Whereas QP, a dopamine D_2/D_3 receptor agonist, inhibited ongoing discharges, Tyr-D-Ala-Gly-*N*-Me-Phe-Gly-ol enkephalin, a μ-opioid receptor agonist, had no effect (data not shown). In addition, DA neurons exhibit a promi-

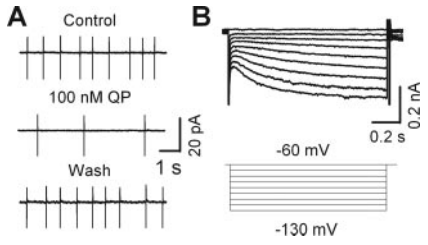

Fig. 1. Dopamine neurons have distinct properties. A, cell-attached recordings show that 100 nM QP (dopamine D_2/D_3 receptor agonist) reversibly depressed a putative DA neuron. B, top, typical whole-cell current traces illustrate I_h evoked in a putative DA neuron by a series of hyperpolarizing voltage pulses (from −70 mV to −130 mV, in steps of 10 mV, as shown below). For this and the following figures, all records were recorded from putative DA neurons mechanically dissociated from SNc.

nent hyperpolarization activated inward current (I_h) in response to a series of voltage steps from −60 to −130 mV (with decrement of 10 mV) when recorded under whole-cell voltage-clamp conditions (Fig. 1B, top). These are characteristic electrophysiological properties of DA neurons. The following experiments were done on putative DA neurons identified according to the aforementioned characteristics.

Mefloquine Enhances the Frequency of GABAergic sIPSCs on DA Neurons. Whole-cell currents were recorded from mechanically dissociated SNc DA neurons. sIPSCs were recorded at a holding potential (V_h) of −50 mV in the presence of APV (50 μM) and DNQX (10 μM), which eliminate glutamate receptor-mediated synaptic transmission. In 21 cells tested under these conditions, bicuculline (10 μM) reversibly abolished all of the spontaneous postsynaptic events, indicating that they were $GABA_A$ receptor-mediated IPSCs (Fig. 2A).

Figure 2B illustrates the effect of mefloquine on sIPSCs of SNc DA neurons. Application of 3 μM mefloquine increased sIPSC frequency by 100 ± 10% ($p < 0.001$, $n = 5$). This is further illustrated in B_2 by the significant leftward shift of the cumulative probability plots of the intervals between successive sIPSCs. As illustrated in Fig. 2C, mefloquine-induced enhancement was fast and reversible and depended on its concentrations. The concentration-response relationship in Fig. 2D shows that the threshold concentration of mefloquine-induced enhancement of sIPSC frequency was between 0.1 and 0.3 μM. Mefloquine (0.3 μM) significantly increased sIPSC frequency by 32 ± 12% ($p < 0.05$, $n = 5$). Mefloquine-induced enhancement was saturated between 3 and 10 μM. Mefloquine (10 μM) enhanced sIPSC frequency by 110 ± 20% ($p < 0.001$, $n = 5$). At concentrations of 0.1 and 1 μM, mefloquine increased sIPSC frequency by 10 ± 10% ($p > 0.05$, $n = 6$) and 43 ± 10% ($p < 0.001$, $n = 6$), respectively. Fit of a logistic equation to these data yielded an estimated EC_{50} of 1.3 μM.

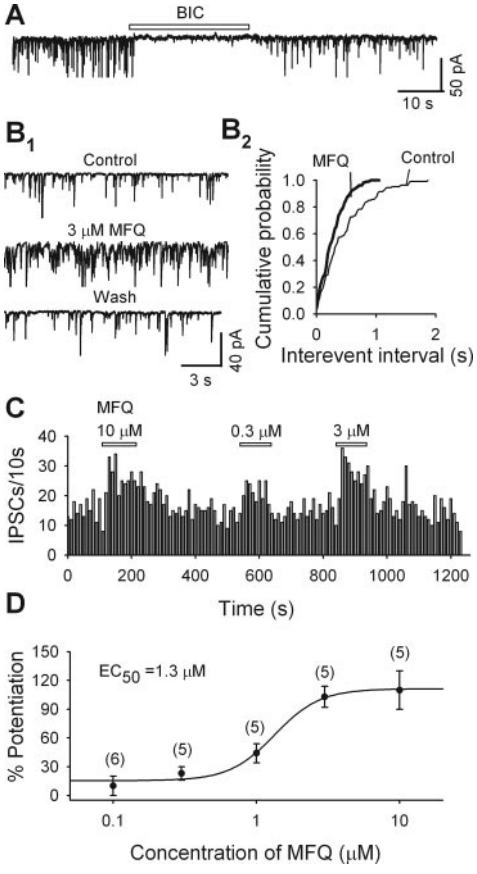

Fig. 2. Mefloquine (MFQ) potentiates sIPSCs. A, sIPSCs recorded from a putative DA neuron were completely (but reversibly) blocked by 10 μM BIC. For this and the following figures, all IPSCs were recorded in the whole-cell configuration at a holding potential of −50 mV, in the presence of APV (50 μM) and DNQX (10 μM). B_1, application of 3 μM mefloquine dramatically and reversibly enhances the frequency of sIPSCs. B_2, cumulative probability plots show much increased incidence of short sIPSC interevent intervals (K-S test, $p < 0.001$; 3 μM mefloquine versus control). C, time course of increases in sIPSC frequency by 10, 0.3, and 3 μM mefloquine (in one cell). For this and the following figures, the open bars above indicate the time courses of the application of the chemicals indicated. D, dose-dependent potentiation of sIPSC frequency by mefloquine (0.1–10 μM; with an EC_{50} of 1.3 μM). Numbers of cells are indicated in parentheses. Values are mean ± S.E.M. The smooth curve is produced by the fitting of the logistic equation to the data points.

Mefloquine Increases the Frequency of mIPSCs. To determine the location where mefloquine acts, we examined the effect of mefloquine on mIPSCs in the presence of TTX (1 μM) to eliminate action potential-induced spontaneous events. As shown in Fig. 3A, 3 μM mefloquine robustly increased mIPSC frequency. This is further illustrated in Fig. 3C by the significant leftward shift of the cumulative probability plot of the intervals between successive mIPSCs, as well as by the accompanying histogram (K-S test, $p < 0.01$). In five neurons tested, 3 μM mefloquine increased the frequency of mIPSCs by 130 ± 10% ($p < 0.001$). In contrast, 3 μM mefloquine did not change the mean amplitude of the mIPSCs (Fig. 3C, right, K-S test, $p = 0.8$). The mean amplitude of mIPSCs in the presence of mefloquine was 101 ± 5% of control ($p > 0.05$, $n = 5$).

Role of Ca^{2+} in Mefloquine-Induced Enhancement of sIPSC Frequency. To assess the contribution of voltage-gated calcium channels (VGCCs), we compared the effects of mefloquine (3 μM) in the absence and presence of Cd^{2+} (100

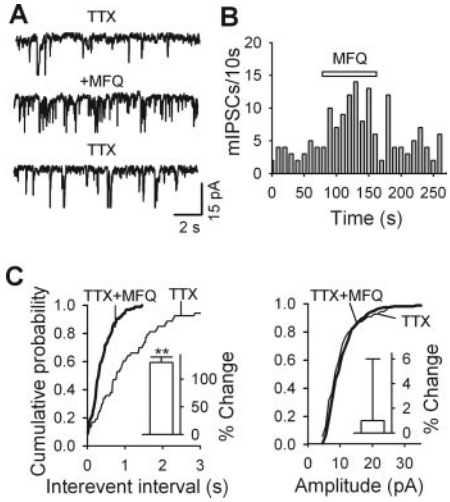

Fig. 3. Mefloquine (MFQ) increases the frequency of mIPSCs. A, sample traces of GABAergic mIPSCs that were recorded before, during, and after application of 3 μM mefloquine in the presence of 1 μM TTX. B, time course of increase in mIPSC frequency induced by 3 μM mefloquine (in one cell). C, mefloquine (3 μM) caused a significant leftward shift of the cumulative probability plot for interevent interval (left, **, $p < 0.001$, K-S test) but not for the amplitude (right, $p > 0.05$, K-S test) of GABAergic mIPSCs. Insets, pooled data from five neurons show that mefloquine increases mIPSC frequency but not amplitude.

μM), a nonselective VGCC blocker. Mefloquine enhanced sIPSC frequency by 113 ± 20% ($n = 5$, $p < 0.001$) in the absence of Cd^{2+} and by 94 ± 11% ($n = 5$, $p < 0.001$) in the presence of Cd^{2+}. Because these two values are equivalent ($p > 0.05$, $n = 5$) (Fig. 4, A and D), mefloquine-induced potentiation of GABA release was not dependent on VGCCs.

To determine whether Ca^{2+} influx was required in the action of mefloquine on sIPSCs, we compared the effect of mefloquine (3 μM) in normal medium and in medium containing a lower Ca^{2+} concentration. Mefloquine (3 μM) enhanced sIPSC frequency by 113 ± 20% in normal medium containing 2 mM Ca^{2+}, but only by 36 ± 9% in medium containing 0.5 mM Ca^{2+} ($p < 0.05$, $n = 5$) (Fig. 4, B and D). This indicates that mefloquine-induced potentiation of GABA release was dependent on extracellular Ca^{2+}.

To test whether intraterminal Ca^{2+} contributes to the facilitation of mefloquine on sIPSC frequency, we examined the effect of BAPTA-AM, a membrane-permeable Ca^{2+} chelator. Approximately 60 to 80 min after pretreatment with 30 μM BAPTA-AM, 3 μM mefloquine increased sIPSC frequency by 21 ± 11% ($p = 0.3$, $n = 6$) (Fig. 4, C and D). Thus, mefloquine failed to increase sIPSC frequency after pretreatment with BAPTA-AM. These observations suggest that mefloquine-induced potentiation of IPSC frequency requires an increase in Ca^{2+} concentration within the presynaptic terminals.

Physostigmine Attenuates Mefloquine-Induced Potentiation of sIPSC Frequency. It has been reported that mefloquine inhibits cholinesterase (Lim and Go, 1985; McArdle et al., 2005). Therefore, we next explored whether inhibition of cholinesterases with physostigmine can attenuate mefloquine-induced potentiation of sIPSC frequency. As shown in Fig. 5, 30 μM physostigmine (PHY) alone increased sIPSC frequency by 50 ± 15% of control ($p < 0.05$, $n = 5$). After the response to physostigmine had stabilized, the application of mefloquine continued to significantly enhance sIPSC frequency by 31 ± 12% ($p < 0.05$, $n = 5$). However, this increase is much smaller than the enhancement induced by mefloquine alone (90 ± 10%) ($p < 0.05$, $n = 5$). This suggests that mefloquine-induced enhancement of sIPSC frequency partially depends upon its anticholinesterase action. Additional mechanisms, including mobilization of intracellular

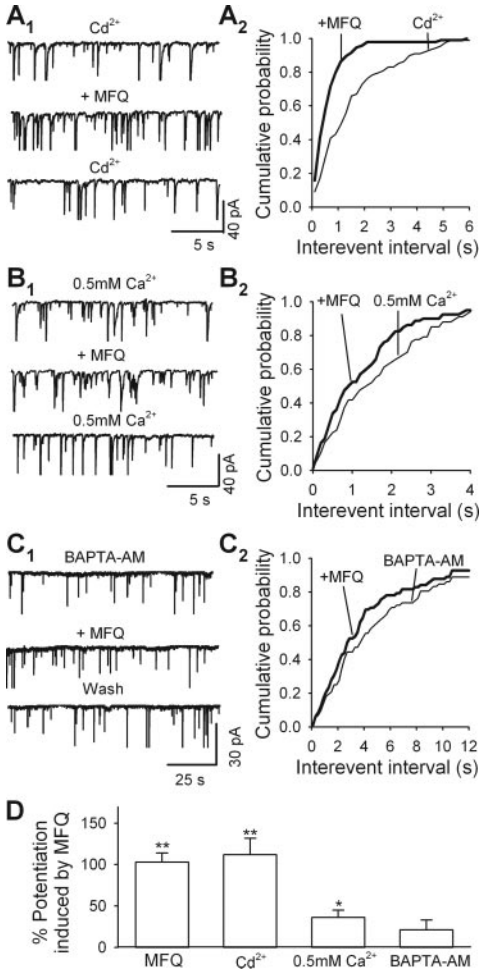

Fig. 4. Intra- and extracellular Ca^{2+} but not VGCCs are involved in mefloquine (MFQ)-induced potentiation of sIPSC frequency. GABAergic sIPSCs were recorded before, during, and after application of 3 μM mefloquine in the presence of Cd^{2+} (A_1) or Ca^{2+} (0.5 mM) (B_1) and pretreated with BAPTA-AM (30 μM) for 60 to 80 min (C_1). Cumulative probability plots for interevent intervals are shown in A_2 ($p < 0.001$, K-S test), B_2 ($p < 0.05$, K-S test), and C_2 ($p > 0.05$, K-S test). D, summary of the effects of 3 μM mefloquine on sIPSC frequency in medium containing 2 mM Ca^{2+} (MFQ), 0.5 mM Ca^{2+}, with 100 μM Cd^{2+} added, and with 30 μM BAPTA-AM added. *, $p < 0.05$; **, $p < 0.001$ compared with the control group.

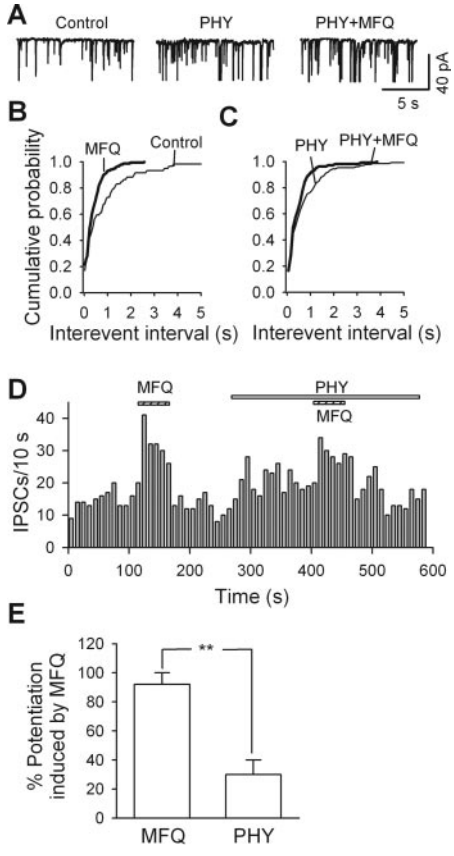

Fig. 5. Mefloquine-induced facilitation of sIPSCs involves in the inhibition of cholinesterases. A, sample traces show that the cholinesterase inhibitor PHY (30 μM) enhances sIPSC frequency but attenuated mefloquine (MFQ, 3 μM)-induced enhancement of sIPSC frequency. Cumulative probability plots for the interevent interval of IPSCs from one cell show the effect of mefloquine applied alone (B) and in the presence of physostigmine (C). D, time course of the changes in sIPSC frequency induced by mefloquine in the absence and presence of 30 μM physostigmine from one cell. E, summary of 3 μM mefloquine-induced enhancement of sIPSC frequency in the absence (MFQ) and presence of 30 μM PHY ($n = 6$). Note that in the presence of physostigmine, the effect of mefloquine is significantly attenuated. **, $p < 0.001$.

Ca^{2+}, are likely to mediate the remainder of the increase of sIPSC frequency (McArdle et al., 2006).

Presynaptic nAChRs Are Involved in Mefloquine-Induced Potentiation of sIPSC Frequency. It is known that when acetylcholinesterase (AChE) is inhibited, acetylcholine accumulates and activates more AChRs. In addition, previous studies have reported the presence of several subtypes of nAChRs, including the α7 and non-α7 nAChRs in the presynaptic sites of midbrain DA neurons (Wonnacott, 1997; MacDermott et al., 1999; Mansvelder and McGehee, 2000). To further test whether mefloquine enhanced GABA release by its anticholinesterase action, we examined the contribution of α7 and non-α7 nAChRs to mefloquine-induced facilitation of sIPSC frequency.

After more than 10 min of pretreatment with α-BgTX (300 nM), a specific α7 nAChR antagonist, sIPSC frequency was not significantly altered (95 ± 6% of control, $p = 0.21$, $n = 7$, data not shown). Mefloquine (3 μM) enhanced sIPSC frequency by 113 ± 12% ($n = 7$, $p < 0.01$) in the absence of α-bungarotoxin and by 118 ± 4% ($p < 0.01$, $n = 6$) in the presence of 300 nM α-bungarotoxin (Fig. 6, A and D). Because these two values are equivalent ($p > 0.5$, $n = 6$), mefloquine-induced potentiation of GABA release was independent of α7 nAChRs.

After a 5-min preincubation with MEC (10 μM), a non-α7 nAChR antagonist, sIPSC frequency was depressed by 20 ± 4% ($p < 0.01$, $n = 6$). Subsequent application of mefloquine increased sIPSC frequency by only 30 ± 9% ($p < 0.01$, $n = 6$), which was significantly less than the increase in the absence of MEC (Fig. 6, B and D). Likewise, the application of DHβE (100 nM), an antagonist for nAChRs containing $\alpha4\beta2$ subunits, depressed sIPSC frequency by 30 ± 4% ($p < 0.01$, $n = 6$). In the absence of DHβE, mefloquine (3 μM) enhanced sIPSC frequency by 113 ± 12% ($p < 0.01$, $n = 6$). In contrast, after a 5-min preincubation in DHβE (100 nM), mefloquine (3 μM) increased sIPSC frequency by only 28 ± 8% ($p < 0.05$, $n = 6$) (Fig. 6, C and D). These results indicate that presynaptic nAChRs containing $\alpha4\beta2$ subunits are involved in mefloquine-induced potentiation of sIPSC frequency.

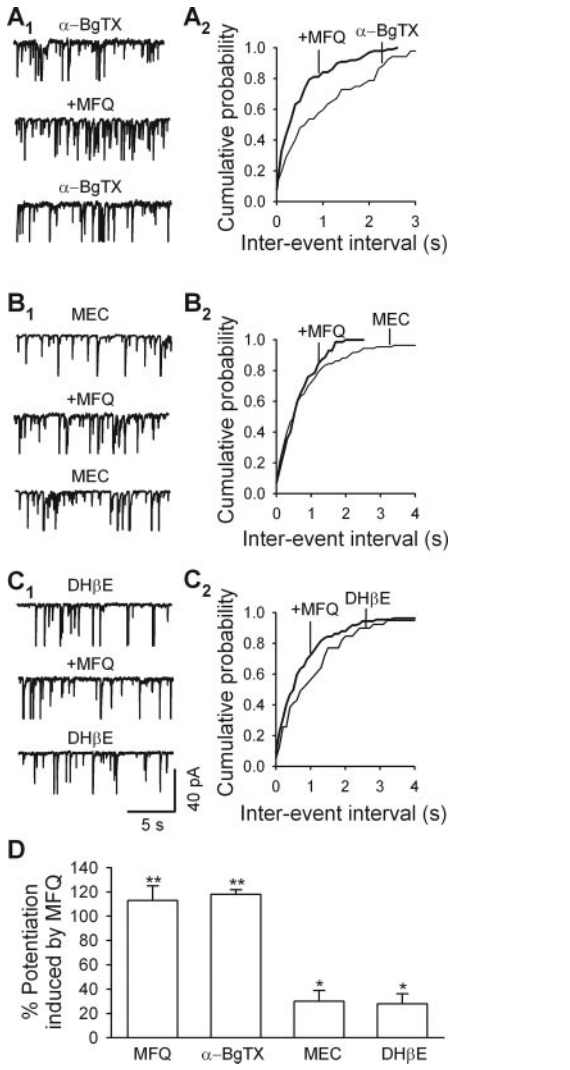

Fig. 6. Mefloquine-induced potentiation of sIPSC frequency involves presynaptic nAChRs. GABAergic sIPSCs were recorded before, during, and after application of 3 μM mefloquine in the presence of α-BgTX (300 nM) (A_1), MEC (10 μM) (B_1), and DHβE (100 nM) (C_1). Cumulative probability plots for interevent intervals are shown in A_2 ($p < 0.05$, K-S test), B_2 ($p < 0.05$, K-S test), and C_2 ($p < 0.001$, K-S test). D, summary of the effect of 3 μM mefloquine on sIPSC frequency in the absence (MFQ) and presence of α-BgTX, MEC, and DHβE ($n = 6$–7).

Discussion

Mefloquine Enhances GABA Release onto Midbrain DA Neurons. Our major finding is that mefloquine enhanced $GABA_A$ receptor-mediated synaptic transmission via the inhibition of AChE. Mefloquine concentration-dependently enhanced sIPSC frequency with an EC_{50} of 1.3 μM. It significantly enhanced sIPSC frequency at a concentration of 0.3 μM. The maximal enhancement reached 110%. It should be emphasized that this effect of mefloquine is very potent, considering that the plasma concentrations are estimated to be 3.8 to 23 μM during mefloquine therapy (Simpson et al., 1999; Kollaritsch et al., 2000). Furthermore, mefloquine raises the frequency of spontaneous mIPSCs in the presence of tetrodotoxin, without altering their mean amplitude. These data suggest that mefloquine acts at the presynaptic site to increase GABA release.

Role of Ca^{2+} in Mefloquine-Enhanced GABA Release. Ca^{2+} influx into the terminals through VGCCs is a common mechanism of modulation of transmitter release. Mefloquine blocks L-type VGCCs as well as volume- and calcium-activated chloride channels in crude microsomes prepared from brain (Lee and Go, 1996). However, mefloquine-induced enhancement of sIPSC frequency did not change in the presence of Cd^{2+} under our experimental conditions. This indicates that VGCCs are not involved in mefloquine-induced enhancement of sIPSCs. Interestingly, a decrease in extracellular Ca^{2+} attenuated mefloquine-induced facilitation of GABA release. This indicates that the action of mefloquine depends on extracellular Ca^{2+}. The residual Ca^{2+} in nerve terminals is known to influence transmitter release (Creager et al., 1980; Augustine et al., 1987; Mennerick and Zorumski, 1995; Debanne et al., 1996; Sullivan, 1999). In the presence of the high-affinity membrane-permeable Ca^{2+} chelator BAPTA-AM, which can efficiently buffer intraterminal Ca^{2+}, mefloquine-induced enhancement of GABA release was almost eliminated. This is consistent with previous findings at the neuromuscular junction (McArdle et al., 2006). Taken together, mefloquine enhances GABA release by increasing Ca^{2+} entry into GABAergic ter-

minals via pathways independent of VGCCs. However, because in low Ca^{2+} medium mefloquine still significantly enhanced GABA release, other pathways independent of extracellular Ca^{2+}, such as inhibition of Ca^{2+} uptake into mitochondria (Lee and Go, 1996; McArdle et al., 2006), may also be involved in the action of mefloquine.

Anticholinesterase Activity and Presynaptic nAChRs Mediate Mefloquine-Induced Enhancement of GABA Release. The SNc DA neurons receive cholinergic input from the pedunculopontine nucleus (Lichtensteiger et al., 1982; Clarke et al., 1985; Swanson et al., 1987; Bolam et al., 1991). Both AChE and nAChRs are expressed in SNc (Henderson and Greenfield, 1984; Emmett and Greenfield, 2005). ACh released from cholinergic terminals activates nAChRs to induce influx of cations and excitation of dopaminergic neurons in SNc. AChE hydrolyzes ACh and terminates the action of ACh.

Both non-α7 and α7 nAChRs are expressed in midbrain. However, in SNc, $\alpha4\beta2$ nAChRs express at high density. In contrast, α7 nAChRs are at low density (Wooltorton et al., 2003). Nicotinic AChRs present on presynaptic terminals facilitate the release of many neurotransmitters, such as GABA, glutamate, serotonin, and dopamine (McGehee et al., 1995; Wonnacott, 1997; MacDermott et al., 1999). In the present study in SNc DA neurons, MEC, a non-α7 nAChR antagonist, and DHβE, a selective antagonist of $\alpha4\beta2$ nAChRs, but not α-bungarotoxin, a selective antagonist of α7 nAChRs, depressed basal sIPSC frequency. These findings suggest the non-α7 nAChRs on GABAergic terminals are tonically activated. Because of its anticholinesterase action (Lim and Go, 1985; McArdle et al., 2005), mefloquine may enhance GABA release via the activation of presynaptic nAChRs. In support of this hypothesis, mefloquine-induced enhancement of GABA release was attenuated in the presence of physostigmine. Thus, it is conceivable that mefloquine inhibits AChE, which allows accumulation of ACh. The resultant activation of nAChRs on GABAergic terminals facilitates GABA release. We attempted to identify the possible combinations of nAChR subunits on GABAergic terminals on rat SNc DA neurons. MEC and DHβE, but not α-bungarotoxin, reduced mefloquine-induced facilitation of sIPSC frequency. Therefore, the presynaptic nAChRs involved in the action of mefloquine are likely to correspond to a class of heteroligomers containing $\alpha4\beta2$ subunits.

In conclusion, our data suggest that mefloquine enhances GABA release through its inhibition of cholinesterase. This allows accumulation of endogenously released acetylcholine, which activates neuronal nicotinic receptors, probably the $\alpha4\beta2$ nAChRs on GABAergic nerve terminals. The resultant increase of Ca^{2+} entry into these GABAergic terminals enhances vesicular release of GABA. This action may contribute to the neurobehavioral effects of mefloquine given that it occurred at the concentrations (0.3–10 μM) equivalent to or even below the plasma concentrations (3.8–23 μM) during mefloquine therapy.

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