Effects of Quinine, Quinidine and Chloroquine on Human Muscle Nicotinic Acetylcholine Receptors

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The genus Cinchona is known for a range of alkaloids, such as quinine, quinidine, cinchonine, and cinchonidine. Cinchona bark has been used as an antimalarial agent for more than 400 years. Quinine was first isolated in 1820 and is still acknowledged in the therapy of chloroquine-resistant falciparum malaria; in lower dosage quinine has been used as treatment for leg cramps since the 1940s. Here we report the effects of the quinoline derivatives quinine, quinidine, and chloroquine on human adult and fetal muscle nicotinic acetylcholine receptors (nAChRs). It could be demonstrated that the compounds blocked acetylcholine (ACh)-evoked responses in Xenopus laevis oocytes expressing the adult nAChR composed of αβεδ subunits in a concentration-dependent manner, with a ranked potency of quinine (IC50 = 1.70 µM), chloroquine (IC50 = 2.22 µM) and quinidine (IC50 = 3.96 µM). At the fetal nAChR composed of αβγδ subunits, the IC50 for quinine was found to be 2.30 µM. The efficacy of the block by quinine was independent of the ACh concentration. Therefore, quinine is proposed to inhibit ACh-evoked currents in a non-competitive manner. The present results add to the pharmacological characterization of muscle nAChRs and indicate that quinine is effective at the muscular nAChRs close to therapeutic blood concentrations required for the therapy and prophylaxis of nocturnal leg cramps, suggesting that the clinically proven efficacy of quinine could be based on targeting nAChRs.

Keywords: quinine, nocturnal leg cramps, nicotinic acetylcholine receptor, two-electrode voltage clamp, ion channel block

INTRODUCTION

Quinoline derivatives such as natural quinine, quinidine, and synthetically produced chloroquine are well known for their use in the treatment of malaria (Figure 1). The efficacy of quinine against nocturnal leg cramps has been proven in randomized clinical trials (Diener et al., 2002; El-Tawil et al., 2015). But the muscle relaxant mechanism of action of quinine has not been fully elucidated yet. As it does not freely cross the blood brain barrier (Silamut et al., 1985), quinine is supposed to be a peripheral muscle relaxant in vivo. In higher dosage as used against malaria, central nervous effects may occur. Quinine and its derivatives are acting on a variety of ion channels including several types of potassium channels (Glavinović and Trifaro, 1988; Imai et al., 1999), members of family of ligand-gated ion channels such as the 5-HT3-type of serotonin receptor (Thompson et al., 2007; Thompson and Lummis, 2008) and nicotinic acetylcholine receptors (nAChR).
Quinine in the therapy and prophylaxis of leg cramps. This effect is possibly the basis for their use in further applications like the treatment of muscle cramps (Katzberg, 2015) and slow channel congenital myasthenic syndrome (SCCMS) (Harper and Engel, 1998; Harper et al., 2003; Peyer et al., 2013).

It is known that quinine acts on muscular and neuronal nAChRs (Sieb et al., 1996; Fukudome et al., 1998a,b; Ballestero et al., 2005). However, the interaction of quinine has also been reported for receptors present at the neuromuscular junction, in which it produces long-lived open-channel as well as a closed-channel block and can normalize the open duration of channel events in the slow-channel congenital myasthenic syndrome (Sieb et al., 1996; Fukudome et al., 1998a,b). Slow channel congenital myasthenic syndrome (SCCMS) is caused by missense mutations in subunits of nicotinic acetylcholine receptor (AChR) at the neuromuscular junction (Engel, 2018). Mutated AChR channel produces prolonged opening events leading to a depolarization block and endplate myopathy. Quinoline derivatives such as quinidine correct the prolonged opening times of the mutated acetylcholine receptor channels in myasthenic syndrome (Bleecker et al., 1998). On the contrary, in myasthenia gravis quinine is contraindicated because it decreases the excitability of the motor end-plate region, thereby reducing responses to repetitive nerve stimulation by acetylcholine. These may be the underlying effects explaining the use of low dose quinine in the therapy and prophylaxis of leg cramps.

Quinine is also blocker for the neuronal α9α10 nAChRs. At this cochlear type of ACh receptor, a mixed competitive and non-competitive inhibition was observed (Ballestero et al., 2005). nAChRs form together with 5-HT3-, GABA(A)-, and glycine receptors the superfamily of ligand-gated ion channels (Albuquerque et al., 2009). Possibly due the strong structural and functional similarities between these receptors, quinine is also a blocker of 5-HT3- and at elevated concentrations also of GABA(A) receptors (Thompson and Lummis, 2008).

The composition of muscle nAChRs is dependent on the developmental stage. The fetal nAChRs together with the receptor in denervated muscles is composed of αβγδε subunits with a stoichiometry of (α1)2β1γδε. In the adult, γ is replaced by the ε subunit (Kalamida et al., 2007). In our present study, we examined the effects of the quinoline derivatives quinine, quinidine, and chloroquine on adult and fetal human muscle nAChRs recombinantly expressed in X. laevis oocytes and provide evidence that these compounds block the nAChRs.

**MATERIALS AND METHODS**

**Expression System**

The expression plasmids contain the cDNA coding for the different human nAChR subunits in pRBG4 (Ohno et al., 1996). Cloned muscular AChR subunit cDNAs were kindly provided by Dr A. G. Engel (Mayo Clinic, Rochester, MN, United States) and Dr. F. Grassi (Carattere Scientifico San Raffaele Pisana, Rome, Italy). Cloned cDNA of the human α7 AChR subunit was described by Schreiner et al. (2014). cRNAs were prepared using the AmpliCap T3 high-yield message marker kit (Epicenter, Madison, WI, United States), following the manufacturer’s protocol. Oocytes were obtained as previously described (Gisselmann et al., 2004) and injected with a total amount of ~30 ng of a mixture of the receptor-coding RNA using an injection-setup from WPI (Nanoliter 2000, Micro4). For the muscular type, a mixture of cRNAs for α, β, δ, ε, and γ in a stoichiometry of 2:1:1:1 was used and for the fetal type, ε was replaced by γ. The injected oocytes were stored in ND 96 (96.0 mM NaCl, 2.0 mM KCl, 1.8 mM CaCl2, 1.0 mM MgCl2, 5.0 mM HEPES, pH 7.2, 200 U/ml penicillin, and 200 µg/ml streptomycin) at 12 °C.

**Electrophysiology**

The electrophysiological recordings were performed using the two-electrode voltage clamp technique as previously described (Sergeeva et al., 2010). All of the measurements were performed in normal frog ringer (NFR) [115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl2, 10 mM HEPES; pH 7.2 (NaOH/HCl)]. The currents were recorded at a holding potential of typically -60 mV using the CellWorks 6.1.1. software (NPI).

**Substances**

The chemicals were obtained from Sigma-Aldrich or Cassella-med GmbH & Co. KG (quinine) and disssolved in NFR.

**Data Analysis**

In blocking experiments, the test substances were applied in an alternating manner with ACh. Therefore, the currents of the test substances or the modulated currents were normalized to the mean of the ACh-induced currents before and after the test substance was applied. The concentration-response data were fitted with the logistic equation using SigmaPlot 8.0 (SPSS). The deviations are represented by the standard error of the mean (SEM).

**RESULTS**

**Effect of Quinoline Derivatives on Adult Muscle nAChRs**

For the pharmacological characterization, we expressed the human muscle nAChRs recombinantly in X. laevis oocytes and characterized the ion channel activity using the two-electrode voltage-clamp technique. In the first experiments, we established a concentration response curve for ACh on Xenopus oocytes expressing the adult nAChR composed of αβγδε subunits (Figure 2). Under our experimental conditions, an EC50 value for ACh of 12.65 ± 2.40 μM, nH = 1.77 ± 0.05 (n = 6) was determined, similar to the EC50 values previously reported in this expression system (Jonsson Fagerlund et al., 2009). Next,
we tested the modulatory effects of quinoline derivatives on the response evoked by 10 μM ACh, a near EC₅₀ value of the agonist. All three tested quinoline derivatives blocked the ACh-evoked currents in a dose-dependent manner with a potency in the low μM range: quinine was most potent with an IC₅₀ of 1.70 ± 0.12 μM (n = 6) followed by chloroquine with an IC₅₀ of 2.22 ± 0.19 μM (n = 6). Quinidine was somehow less potent and blocked with an IC₅₀ of 3.96 ± 0.36 μM (n = 6) (Figure 3). The block by quinine was reversible, as shown in the Supplementary Figure 1A.

**Non-competitive Action of Quinine**

In the rat α9α10 nAChRs, a mixed competitive / non-competitive mode of antagonism for quinine was observed (Ballestero et al., 2005). To determine the mode of antagonism for quinine in the adult muscle nAChRs, we established concentration-response curves for ACh alone and for different concentrations of ACh in the presence of quinine at the same Xenopus oocyte. We used 1.8 μM quinine which is a near IC₅₀ concentration. In the case of a non-competitive mechanism, the inhibition should be independent of the ACh concentration, whereas the efficacy of competitive antagonists decreases with increasing ACh concentrations. The efficacy of quinine was independent of the ACh concentration (Figure 4A) and the determined EC₅₀ of ACh didn’t significantly change in the presence of 1.8 μM quinine (Figure 4B). Further, also at elevated ACh concentrations, the maximally evoked current was never reached in the presence of 1.8 μM quinine (Figure 4B).

For the rat α9α10 nAChRs a slight voltage dependency of the quinine block was described indicating a potential binding site within or near the channel pore (Ballestero et al., 2005). We tested whether the quinine block of the muscular AChR was also voltage dependent and found that 1.8 μM quinine blocked more pronounced at negative (−60 mV, I/Iₘₐₓ = 0.45 ± 0.11,
Effect of Quinine on Fetal Muscle and Neuronal nAChRs

For the pharmacological characterization, we expressed the fetal muscle nAChRs composed of αβγδ subunits recombinantly in X. laevis oocytes and characterized the ion channels using the two-electrode voltage-clamp technique. In the first experiments, we established a concentration response curve for ACh on Xenopus oocytes expressing fetal nAChRs (Figure 5). Under our experimental conditions, an EC$_{50}$ value for ACh of 7.60 ± 1.87 µM (n = 6) was determined, comparable for the EC$_{50}$ reported for fetal receptors in the Xenopus oocyte system (Garland et al., 1998). Next, we tested the modulatory effects of quinine on the response evoked by 10 µM ACh, a near EC$_{50}$ value of the agonist, which blocked the ACh-evoked currents in a dose-dependent manner with an IC$_{50}$ of 2.30 ± 0.17 µM (n = 3) (Figure 4).

For the pharmacological characterization of neuronal receptor types, we expressed nAChRs composed of α7 subunits in X. laevis oocytes. Under our experimental conditions, the EC$_{50}$ value for ACh was previously reported to be 90.5 µM...
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FIGURE 4 | Ach concentration–response relationship at human adult muscle nAChRs in the presence and absence of 1.8 µM quinine measured in *Xenopus* oocytes. Currents were elicited by various Ach concentrations. (A) Concentration–response curves for human adult muscle nAChRs in the presence (open circles) and the absence (filled circles) of 1.8 µM quinine (*n* = 6). (B) Relative current blocked by 1.8 µM quinine at 10, 30, and 100 µM Ach. Holding potential: −60 mV, error bars represent S.E.M.

(Schreiner et al., 2014). We tested the modulatory effects of quinine on the response evoked by 100 µM Ach, a near EC50 value of the agonist, which blocked the Ach-evoked currents in a dose-dependent manner with an IC50 of 12.8 ± 1.3 µM (*n* = 3) (Supplementary Figure 1B). The neuronal α7 subtype is of significantly lower potency compared to the muscular type (*p* < 0.0001).

DISCUSSION

This study describes the effects of quinine, quinidine, and chloroquine on human muscle nAChRs. All three compounds are potent antagonists which block in the low µ-molar range (IC50 ~ 1.7–4 µM) which corresponds to a concentration of 0.6–1.4 mg/l quinine sulfate. Compared with the reported potency at other receptors, quinine shows a similar potency at the neuronal rat α9α10 nAChRs (IC50 ~ 1 µM) and the human homomeric 5HT3A-receptors (IC50 ~ 1 µM), but is less potent at the human neuronal α7 nAChR (IC50 ~ 13 µM), the human heteromeric 5HT3AB- (IC50 ~ 16 µM) or human GABA(A)-receptors (IC50 ~ 1.6 mM) (Ballestero et al., 2005; Thompson and Lummis, 2008). At a similar low µM concentration range the interference with the neuromuscular transmission (Sieb et al., 1996) or the decrease in the open duration of muscle nAChRs (Fukudome et al., 1998b) was observed for quinoline derivatives in vitro.

At human muscle nAChRs receptors the observed quinine block was consistent with a non-competitive antagonism. These mode of antagonism for quinine was also reported at human heteromeric 5-HT3AB-receptors (Thompson and Lummis, 2008). At rat neuronal α9α10 nAChRs, quinine was proposed to block competitively at lower, however, non-competitively at higher concentrations (Ballestero et al., 2005). We observed a voltage-dependency for the quinine block of the muscular AChR similar to that reported for the rat α9α10 nAChR. Our results indicate that quinine could interact with a charged side chain within or near the channel pore as discussed in detail for the α9α10 nAChR by Ballestero et al. (2005).

Human homomeric 5-HT3A and GABA(A) receptors were blocked in a competitive manner (Thompson and Lummis, 2008). Binding studies at the recombinant muscle receptor expressed in HEK293 cells support the observed non-competitive
antagonism of quinine, as even concentrations up to 30 \( \mu \text{M} \) of the antagonist could not inhibit the binding of \( \alpha \)-bungarotoxin (Sieb et al., 1996).

Regarding the high homology between 5-HT\( _3\)A, GABA(A) and nAChRs and the similarities in the actions of quinoline compounds, conserved sites of action for these compounds were suggested (Thompson and Lummis, 2008).

For the therapy of malaria, an about ten-fold higher oral dosage of quinine is needed than against muscle cramps, and anti-malaria plasma concentrations of 10–15 mg/l are recommended. During acute malaria, quinine plasma concentrations are higher than under non-infectious conditions. Metabolic clearance of quinine in acute illness is reduced because of decreased cytochrome P450 (CYP) 3A4 activity. Increased \( \alpha \)-1-acid glycoprotein concentrations in the acute phases of malaria lead to an increased plasma protein binding of quinine, which contributes to the decreased volume of distribution and the increased quinine concentration during the acute phase of the disease (Kloprogge et al., 2014). Thus, the \textit{in vitro} observed effects of quinine at central nervous receptors are to be expected in general above a plasma concentration of 10 \( \mu \text{g/ml} \) which is not reached after intake of 200–400 mg quinine sulfate against muscle cramps.

When quinine sulfate 2 \( \times \) 260 mg is applied orally to humans, a dosage which lies in the range used in therapy and prophylaxis of nocturnal leg cramps, a peak plasma concentration of 2.5 \( \mu \text{g/ml} \) is reached at about 2 h after intake of quinine. Plasma elimination half time is about 11–12 h; accordingly, plasma concentration was decreased to 1.6 \( \mu \text{g/ml} \) after 12 h (Dieterich et al., 1984). These values are in the same concentration range as measured for the inhibitory effect of quinine at the human muscle AChR; thus, this effect may be postulated as a possible mechanism of action of the muscle relaxant effect of quinine which has been therapeutically used in patients with leg cramps for more than half a century.

In summary, we have shown that the quinine, quinidine and chloroquine antagonize ACh-evoked responses at muscular nAChRs. The potency for quinine at the muscle nAChRs was in the same range as found for neuronal rat \( \alpha 9\alpha 10 \) AChR or human 5-HT\( _3\)A-receptors (Ballestero et al., 2005; Thompson and Lummis, 2008). However, quinine was slightly less potent at the neuronal human \( \alpha 7 \) AChR.

Typical blood and tissue concentrations for quinine indicate possible action on muscle nAChRs. These observations further extend the pharmacological knowledge on receptors affected by quinoline derivatives. The reversible inhibitory effect of quinine on human muscle nAChRs may be one mechanism which contributes to the clinically proven efficacy of quinine against leg cramps. But as quinine is a wide spectrum channel blocker, further research on its interaction with receptors of different type and location would be of interest.

**AUTHOR CONTRIBUTIONS**

HH, GG, and BW-J conceived and designed the experiments. DA performed the experiments. DA and GG analyzed the data. GG and BW-J wrote the paper.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2018.01339/full#supplementary-material

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