Hydroxychloroquine reduces heart rate by modulating the hyperpolarization-activated current I_f: Novel electrophysiological insights and therapeutic potential

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BACKGROUND Bradycardic agents are of interest for the treatment of ischemic heart disease and heart failure, as heart rate is an important determinant of myocardial oxygen consumption.

OBJECTIVES The purpose of this study was to investigate the propensity of hydroxychloroquine (HCQ) to cause bradycardia.

METHODS We assessed the effects of HCQ on (1) cardiac beating rate in vitro (mice); (2) the "funny" current (I_f) in isolated guinea pig sinoatrial node (SAN) myocytes (1, 3, 10 μM); (3) heart rate and blood pressure in vivo by acute bolus injection (rat, dose range 1–30 mg/kg), (4) blood pressure and ventricular function during feeding (mouse, 100 mg/kg/d for 2 wk, tail cuff plethysmography, anesthetized echocardiography).

RESULTS In mouse atria, spontaneous beating rate was significantly ($P < .05$) reduced (by 9% ± 3% and 15% ± 2% at 3 and 10 μM HCQ, n = 7). In guinea pig isolated SAN cells, HCQ conferred a significant reduction in spontaneous action potential firing rate (17% ± 6%, 1 μM dose) and a dose-dependent reduction in I_f (13% ± 3% at 1 μM; 19% ± 2% at 3 μM). Effects were also observed on L-type calcium ion current (I_{CaL}) (12% ± 4% reduction) and rapid delayed rectifier potassium current (I_{Kr}) (35% ± 4%) at 3 μM. Intravenous HCQ decreased heart rate in anesthetized rats (14.3% ± 1.1% at 15mg/kg; n = 6) without significantly reducing mean arterial blood pressure. In vivo feeding studies in mice showed no significant change in systolic blood pressure nor left ventricular function.

CONCLUSIONS We have shown that HCQ acts as a bradycardic agent in SAN cells, in atrial preparations, and in vivo. HCQ slows the rate of spontaneous action potential firing in the SAN through multichannel inhibition, including that of I_f.

KEYWORDS Hydroxychloroquine; Electrophysiology; Heart failure; Arrhythmia; Pacemaker; Heart rate; Ion channels; Funny current; I_f

ABBREVIATIONS **ANOVA** = analysis of variance; **AP** = action potential; **HCQ** = hydroxychloroquine; **HR** = heart rate; I_{CaL} = L-type calcium ion current; I_f = funny current; I_{Kr} = rapid delayed rectifier potassium current; **LV** = left ventricle; **PSS** = physiological saline solution; **SAN** = sinoatrial node; **SBA** = specific bradycardic agent; **SDD** = spontaneous diastolic depolarization; **V50** = voltage of half-activation

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Introduction

Laurent et al[1] described heart rate (HR) as one of the major determinants of myocardial oxygen consumption. Resting HR is an important predictor of cardiac mortality[2] and has emerged as a therapeutic target. Accordingly, agents that reduce HR without affecting ventricular contractility are of major clinical interest for the treatment of ischemic heart disease and heart failure.[3] HR reduction can be achieved with β-adrenoceptor antagonists or rate-limiting calcium channel blockers; however, these agents may exert concomitant negative inotropic and hypotensive effects,[4] potentially exacerbating myocardial ischemia.

In 1987, Kobinger and Lillie[5] described a novel class of substances known as specific bradycardic agents (SBAs), which

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induce sinus bradycardia at a concentration without detrimental hemodynamic effects. SBAs have been shown to reduce cardiac oxygen demand by increasing the diastolic period, which induces an elevation of subendocardial blood flow.[6] The only known SBA on the market, ivabradine (S16257), is an agent that blocks the "funny" current (I_f) and that acts to slow sinus node action potential (AP) firing directly in pacemaking cells.[7]

Interestingly, early work in the late 1950s[8] explored the possibility of using chloroquine or hydroxychloroquine (HCQ) in the treatment of atrial fibrillation (without much understanding of the mode of action), with both drugs demonstrating possible efficacy (although without placebo-group comparison). Since then, there have been case reports of a potential bradycardic action of hydroxychloroquine (HCQ),[3,9,10] which have led us to investigate whether this effect can be observed in cardiac preparations. HCQ was synthesized in 1950 by Surrey and Hammer.[11] Dennis et al[12] showed HCQ to be an immunomodulating agent. It is now widely prescribed for its antimalarial and antirheumatic effects.[13] Here we describe novel SAN-inhibiting properties of HCQ in isolated cardiac preparations and in vivo anesthetized animals. We also investigated the safety of HCQ by in vivo oral feeding on blood pressure and cardiac contractility. The effects of HCQ are consistent across species, from single cells to multicellular preparations. Taken together, our results support the hypothesis that HCQ may be a viable, low-cost pharmacologic agent to reduce HR.

Methods

Animal experiments are described in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines[14] and conform to the Animals (Scientific Procedures) Act 1986 (UK).[15] Procedures were performed under British Home Office license PPL 30/3080.

Isolated cardiac preparations

An expanded Materials and methods section is available in the Supplementary Material online.

Mouse atrial preparations

Hearts were rapidly excised from male CD-1 mice (7–9 wk old) and washed in warm, oxygenated physiological saline solution (PSS). The preparation was hung in a 37°C organ bath filled with PSS. Beating rate was calculated in real-time from the upstroke of the tension signal. Drugs were added cumulatively, directly to the organ bath.

Guinea pig SAN cell isolation

Hearts were excised from male guinea pigs (350– 500 g) and rinsed in modified Tyrode solution. SAN myocytes were then isolated enzymatically.

Single-cell electrophysiology

Perforated patch clamp recordings were carried out, using amphotericin (250 μg/mL) to achieve perforation.

AP recordings

APs were recorded from single guinea pig SAN cells under current clamp conditions.

Voltage clamp recordings

Full current–voltage relations for I_f and L-type calcium ion current (I_{CaL}) as well as rapid delayed rectifier potassium current (I_{Kr}) measurements were taken at 0 minutes and 5 minutes of exposure to HCQ. Please refer to the supplementary material online.

Rat in vivo, invasive hemodynamic studies

HR and arterial blood pressure were measured under general anesthesia in male Sprague Dawley rats (300–350 g), via cannulation of the left carotid artery.

Mice in vivo, noninvasive blood pressure by tail cuff plethysmography and cardiac contractility studies by echocardiography

Automated noninvasive tail cuff plethysmography (Visitech 2000; Visitech Systems, Apex, NC) was used to determine systolic blood pressure in response to HCQ in drinking water, which was compared with that of a control group. Echocardiography was performed at the end of the study in both groups.

Statistics

In vitro statistics

For in vitro statistics, data are presented as means plus or minus standard error (SEM) and analyzed using 1- or 2-way analysis of variance (ANOVA) and with repeated measures where appropriate. Post hoc tests used the Dunnett correction. P values $< .05$ were considered statistically significant.

In vivo statistics

For in vivo statistics, data are presented as means +/- SEM and all data passed a normality test. Within group comparison are made using a 1-way ANOVA, with post hoc analysis to determine significance (Newman–Keuls, $P < .05$). Where in vivo data from 2 groups are compared, these data were analyzed with an unpaired 2-tailed t test.

Results

Bradycardic effects of HCQ on isolated mouse atrial preparation

The application of cumulative doses of HCQ to spontaneously beating mouse atrial preparations revealed a dose-dependent reduction in beating rate ($P < .05$) that was significant at a dose of 3 μM (9% ± 3% slower than control rate, $P < .05$) and further enhanced at 10 μM (15% ± 2% slower than control rate, $P < .05$). Figure 1 demonstrates the beating rate in beats per minute and rate change in relation to control for all concentrations applied. No rate change was seen during time-matched control experiments in which the method was repeated without the addition of HCQ (1.4% ± 2.6% decrease from control in 2 h, $P > .05$, n = 6). Starting