Effects of Chloroquine on Smooth Muscle Contracted with Noradrenaline or High-Potassium Solutions in the Rat Thoracic Aorta

Peter I. AZIBA¹ and D.T. OKPAKO²

¹Department of Pharmacology & Therapeutics, University of Ibadan, and
²Olabisi Onabanjo University Ago Iwoye, Nigeria

Abstract

The effects of chloroquine on the smooth muscle of isolated rat aortic segments were investigated in preparations contracted with either noradrenaline or high-potassium. At rest, chloroquine (up to $10^{-4}$ M) produced no mechanical response, while noradrenaline ($10^{-6}$ M) produced a sustained contraction. In the presence of $10^{-4}$ M chloroquine, however, the amplitude of contractions produced by noradrenaline was attenuated by about 70%, with no alteration of the resting tension. In preparations contracted either with noradrenaline or with high-K solutions, chloroquine produced a concentration-dependent relaxation. The tension decreased below resting level as a result of the co-application of these stimulants. The relaxing actions of chloroquine were not altered by methylene blue (an inhibitor of guanylate cyclase), suggesting that the cyclic GMP-related mechanism was not involved. The ratio of the amplitude of chloroquine-induced relaxation was similar in contractions produced by different concentrations of potassium ions, suggesting that chloroquine did not cause relaxation as a result of membrane hyperpolarization. These results suggest that the inhibition of aortic smooth muscle contraction caused by chloroquine is different to that produced by endothelium-derived vasodilating factors. It is possible that the inhibition of aortic smooth muscle contraction by chloroquine involves modulation of the contractile systems and of their regulatory proteins.

Key words: chloroquine, relaxation, noradrenaline, high potassium, aorta

Introduction

Although chloroquine has been developed primarily as an anti-malarial agent, it is found to be applicable to a wide range of diseases such as inflammation, extraintestinal amebiasis or gout (Isaacson et al., 1982). Clinical treatment with chloroquine is often accompanied by serious side effects such as gastrointestinal upset, pruritus, headache, visual disturbances and cardiotoxic actions (Ekpechi and Okoro, 1964). Experiments using isolated smooth muscle preparations...
from the guinea-pig ileum indicate that chloroquine has an inhibitory action on contractions produced by electrical stimulation (Fameay et al., 1975). Contractions of smooth muscle induced by chloroquine are also demonstrated in different types of mammalian tissue (Ebeigbe et al., 1982; Okpako and Aziba, 1990). The mechanism of action of chloroquine on smooth muscle is equivocal, and there seems to be variation between tissues. In intestinal smooth muscle of the guinea pig, chloroquine does not cause any mechanical response, but inhibits contractions produced by histamine, acetylcholine or 5-hydroxytryptamine, mainly in a non-competitive manner (Olatunde, 1970). However, competitive antagonism of chloroquine with histamine receptors has been demonstrated in smooth muscle of the guinea pig ileum (Akubue, 1975).

The present experiments were carried out to investigate the mechanism of actions of chloroquine on vascular smooth muscle, since circulatory disorders are often associated with administration of chloroquine in malaria therapy (Ekpechi and Okoro, 1964). We measured the effects of chloroquine on smooth muscle contracted with noradrenaline and high-potassium solutions, since there are different cellular mechanisms involved in the contractions produced by these two stimulants in vascular smooth muscle; noradrenaline produces contraction of aortic smooth muscle through activation of α-adrenoceptors, while high-K solutions produce contraction by accelerated influx of $Ca^{2+}$ through voltage-gated $Ca$-channels (Karaki et al., 1997; Kuriyama et al., 1998). Therefore, any difference in the effects of chloroquine on smooth muscle contracted by these stimuli, will assist our understanding of the mechanism of action of chloroquine on vascular smooth muscle. The results indicated that chloroquine causes inhibition of contractions produced by either noradrenaline or high-K solutions to a similar extent, suggesting the possible involvement of voltage-independent and receptor-independent inhibitory actions of chloroquine on contraction of vascular smooth muscle.

**Methods**

The adult albino rats (> 6 weeks old; Sprague Dawley strain) used in these experiments were supplied by the National Institute of Medical Research, Yaba, Lagos, Nigeria. The animals were maintained in the Preclinical Animal House in well ventilated conditions, under constant temperature (30°C) and humidity (50%), and exposed to 12 h light-dark cycle for 2 weeks before use. The animals were fed on standard livestock pellets (Pfizer Nigeria Ltd.), with free access to water, and were treated ethically according to the guidelines for the treatment of experimental animals as determined by the University of Nigeria.

The rats were killed by cutting one of the common carotid arteries during deep anesthesia following a subcutaneous injection of pentobarbital sodium (50 mg/kg). The thoracic aorta was excised and placed in a Petri dish containing a physiological salt solution at room temperature (details below). The vessel was carefully cleaned of adhering connective tissue, and a piece of cotton ball inserted through the lumen to rub off the endothelial cells. Helical strips were then prepared from the vessel, according to the technique used by Furchgott and Bhandarakom (1953). Both ends of each helical strip were tied with fine cotton threads, and mounted in an organ bath (capacity, 10 ml) which was filled with a physiological salt solution maintained at 37°C and aerated with a gas mixture containing 95% $O_2$ and 5% $CO_2$. The preparations were equilibrated
for 1 h in the organ bath in an unstretched condition, and the bathing solution renewed every 20 min. After this equilibration, the contractile responses were recorded isometrically using a force displacement transducer (FT-03, Grass) and the signals amplified with a polygraph (Grass, model 7D).

The physiological salt solution used in the present experiments was a modified Krebs solution (Kosterlitz et al., 1970), with the following ionic composition in mM/L: NaCl 118.0, NaHCO₃ 25.0, KCl 1.2, CaCl₂ 1.6, KH₂PO₄ 1.2, MgCl₂ 1.2 and glucose 25.0. The solution was bubbled with a 95% O₂ - 5% CO₂ gas mixture, which maintained the solution at a pH of between 7.2–7.4. The K⁺-Krebs solution was prepared by replacing NaCl with KCl. The Krebs solution with high potassium concentrations (high-K solution) was prepared by mixing the K⁺-Krebs and normal Krebs solutions, at different ratios. The calcium-free Krebs solution was prepared by deleting CaCl₂ during preparation of the Krebs solution.

Drugs used were acetylcholine bromide (Sigma, St. Louis, MO, USA), chloroquine diphosphate anhydrous (Sigma, St. Louis, MO, USA), noradrenaline hydrochloride (Sigma, St. Louis, MO, USA) and methylene blue (Laboratory HBL Reagent, Nigeria). The stock solution of acetylcholine was prepared by dissolving in a 0.1 M hydrochloric acid solution, while all other solutions were prepared by dissolving in distilled water. The stock solutions were stored at −20°C until use, and then further diluted with Krebs solution to prepare the desired concentrations. This dilution procedure did not alter the pH of the solution.

The data obtained from these experiments were expressed as the mean ± standard error of the mean (S.E.M.), for n observations. The values obtained in the different groups were compared using the Student’s t-test, and probabilities of less than 5% (P<0.05) were considered to indicate a significant difference.

Results

In helical strips of the rat aorta, noradrenaline (10⁻⁶ M) produced a sustained contraction, while chloroquine (up to 10⁻⁴ M) did not produce any mechanical response. In the presence of chloroquine (10⁻⁴ M), however, the amplitude of contractions produced by noradrenaline was strongly attenuated (by about 70%), with no significant alteration to the shape of contraction (Fig. 1, A and B). When chloroquine was added to preparations which had been contracted by noradrenaline for 30 min, a strong relaxation was elicited. The amplitude of this relaxation increased in a concentration-dependent manner, between 10⁻⁵ and 10⁻⁴ M. In response to the addition of 10⁻⁴ M chloroquine, the level of tension went down below the resting value (Fig. 1, C). A similar strong relaxation was elicited when a single concentration (10⁻⁴ M) of chloroquine was added to muscles which had been contracted by noradrenaline (Fig. 1, D).

The effect of methylene blue (an inhibitor of guanylate cyclase) on the chloroquine-induced relaxation was observed in preparations of the rat aorta which had been contracted by noradrenaline. As shown in Fig. 2, the relaxation produced by 10⁻⁴ M chloroquine in the noradrenaline-contracted muscle was not altered in the presence of 10⁻⁵ M methylene blue. This result suggests that the mechanism of relaxation produced by chloroquine may not involve activation of the enzyme guanylate cyclase.
Fig. 1. Effects of chloroquine on contractions produced by noradrenaline in smooth muscle strips of the rat aorta. Preparations were stimulated with $10^{-6}$ M noradrenaline (NA) at filled circle, before (A) and after application of $10^{-4}$ M chloroquine (CQ, B). C. In preparations contracted with $10^{-6}$ M noradrenaline, three increasing concentrations ($10^{-5}$, $4 \times 10^{-5}$, $10^{-4}$ M) of chloroquine (CQ) was applied in a cumulative manner (applied at triangles). D. In preparations contracted with $10^{-6}$ M noradrenaline, $10^{-4}$ M chloroquine was applied at triangle. A-C and D were recorded from different preparations.

Fig. 2. Effects of methylene blue on the relaxation induced by chloroquine in smooth muscle strips of the rat aorta. In preparations contracted with $10^{-6}$ M noradrenaline (NA), $10^{-4}$ M chloroquine (CQ) was applied in the absence (A) and presence of $10^{-5}$ M methylene blue (B). A and B were recorded from the same preparation.

The possible involvement of hyperpolarization in the chloroquine-induced relaxation was evaluated by testing the effects of chloroquine on preparations contracted by different concentrations of high-K solutions. In preparations contracted with solutions containing low (15 mM) and high concentrations (60 mM) of potassium ions, the relationships showing the ratio of relaxation produced by chloroquine ($10^{-5}$–$10^{-4}$ M) were nearly parallel (Fig. 3). These results suggest that the relaxation may be not causally related to the membrane potential of smooth muscle.
Chloroquine-induced aortic relaxation

Fig. 3. Inhibition of contraction produced by high-K solutions in smooth muscle strips of the rat aorta. Smooth muscle strips were contracted by high-K solutions (open circles, 15 mM [K⁺]o solution; filled circles, 60 mM [K⁺]o solution), and increasing concentrations (10⁻⁵, 6 × 10⁻⁵ M, 10⁻⁴ M) of chloroquine were added in a cumulative manner. The amplitude of relaxation elicited by chloroquine was measured as relative to the amplitude of contractions produced by high-K solutions. Mean ± S.E.M. (n=5).

Discussion

The present experiments indicate that in smooth muscle isolated from the rat aorta, chloroquine inhibited contractions produced by either noradrenaline or high-K solutions, in a concentration-dependent manner. The cellular mechanism of contraction involved in the response of arterial smooth muscle to noradrenaline and high-K solution is different. The former is produced mainly by receptor-mediated supply of Ca²⁺ and the latter by influx of Ca²⁺ through voltage-gated Ca-channels (Karaki et al., 1997). As chloroquine inhibited the contraction of aortic smooth muscle produced by these two mechanisms to a similar extent, the blockade of voltage-gated Ca-channels may not be an important factor in the relaxation induced by chloroquine. That is, the inhibitory actions of chloroquine may occur in a voltage-independent manner in smooth muscle cells of the rat aorta.

Endothelial cells lining blood vessels produce an endothelium-derived relaxing factor (EDRF) in response to many types of stimuli such as chemical agents and mechanical stimulation (Furchgott, 1984; Vanhoutte et al., 1986; Moncada et al., 1992). Nitric oxide (NO)
may be the chemical involved, and its diffusion to vascular smooth muscle cells appears to activate soluble guanylate cyclase to enhance the production of cyclic GMP (Moncada et al., 1992). The elevated cyclic GMP in vascular smooth muscle cells causes a relaxation of smooth muscle due to reduction of cytosolic Ca\(^{2+}\) through activation of Ca-ATPase distributed in the membrane of internal stores (Karaki et al., 1997). Methylene blue blocks the actions of EDRF through inhibition of guanylate cyclase (Furchgott, 1984; Vanhoutte et al., 1986; Moncada et al., 1992). The present experiments showed that the relaxation produced by chloroquine is little affected by methylene blue, suggesting that the relaxation does not involve elevated production of cyclic GMP.

Hyperpolarization of the membrane is also one of the important factors to induce relaxation in vascular smooth muscle, and many types of physiological stimulus induce vasodilatation in an endothelium-dependent manner, due to hyperpolarization of the membrane through the release of an endothelium-derived hyperpolarizing factor (EDHF) (Suzuki and Chen, 1990). Vasodilatation is also induced by a group of chemicals known as K-channel openers, through activation of ATP-sensitive K-channels (Cook et al., 1988). The inhibition of noradrenaline-induced contraction by K-channel openers may be mainly due to reduced production of inositol trisphosphate (IP\(_3\)) (Itoh et al., 1992), and this may be also the case for the EDHF-induced vasodilatation. As the chloroquine-induced relaxation observed in aortic smooth muscle preparations which have been contracted with high-K solutions are elicited independently of the concentration of potassium ions, the possible involvement of hyperpolarization due to activation of K-channels is not likely in chloroquine-induced relaxation.

In aortic smooth muscle of the rat, the inhibitory effects of chloroquine on contraction differed depending on when this chemical was applied. Application of chloroquine in preparations which had been contracted by noradrenaline elicited greater inhibitory actions than chloroquine pretreatment prior to noradrenaline application. The mechanism causing this difference remains unclear. In the absence of noradrenaline, chloroquine did not produce any change in the tension of smooth muscle, suggesting that chloroquine itself does not have direct actions on contractile proteins. Alternatively, chloroquine alone was insufficient to inhibit noradrenaline contraction of aortic smooth muscle. The possible requirement for the coexistence of unidentified factors being involved in the chloroquine-induced inhibition must be considered.

The present experiments have shown that relaxation of aortic smooth muscle contracted with noradrenaline or high-K solutions in response to chloroquine is not directly related to the increased production of cyclic GMP or to hyperpolarization of the membrane. Although the detailed mechanism of actions of chloroquine still remains unclear, the effects of this chemical may be directed towards contractile systems within the smooth muscle cells, possibly at the level of regulatory proteins. The different inhibitory actions of chloroquine on noradrenaline-induced contraction between pretreatment and post-treatment application of noradrenaline also remains unclear. Additional experiments related to the effects of chloroquine on contractile systems such as the alteration of calcium sensitivity or regulatory proteins are required.
References


(Received March 1, 2003; Accepted April 28, 2003)