Between 300 to 500 million cases of malaria are reported yearly, which has resulted in over one million deaths annually (1). Also, about two billion people (approximately 40% of the world’s population) in more than 100 countries are at risk of the disease (2). Ninety percent of the cases of malaria occur in children in Africa under 5 years of age (2). The incidence of malaria is made worse in most sub-Saharan Africa because of the emergence of drug-resistant strains of *plasmodium falciparum* which rendered some traditional antimalarial drugs especially the first-line drugs like chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) ineffective. The spread of CQ-resistance has led to an increasing use of amodiaquine (AMQ).

AMQ, chemically known as 4-[(7-chloroquinolin-4-yl)amino]-2-[(diethylamino)methyl]phenol (Figure 1), is an alternative first-line drug for uncomplicated malaria (3). AMQ has been reported to remain remarkably effective in many malaria endemic countries and in particular in West Africa despite emerging CQ resistance (4–9). AMQ either as monotherapy or Artemisinin-based combination therapy (ACT) has proved highly effective in a number of field trials (10, 11).

**Keywords:** Ca²⁺ influx, K⁺ channels, amodiaquine, mesenteric arteries, vasorelaxation

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**VASORELAXATION INDUCED BY AMODIAQUINE IN RAT SUPERIOR MESENTERIC ARTERIES: IN VIVO AND IN VITRO STUDIES**

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**Abstract:** The aim of this study was to investigate the mechanisms underlying vasorelaxation induced by amodiaquine (AMQ) in rat superior mesenteric arteries. In normotensive, conscious rats, AMQ at 1–20 mg/kg i.v. produced hypotension and dose-dependent bradycardia. In mesenteric rings pre-contracted with phenylephrine (PHE) (10⁻⁵ M), AMQ caused concentration-dependent relaxation [IC₅₀ = (1.34 ± 0.04) × 10⁻⁵ M, Eₘₐₓ = 67.5 ± 0.8%]. Vasorelaxation induced by AMQ was unaffected after removal of the endothelium (Eₘₐₓ = 66.9 ± 0.3%, p > 0.05), and in the presence of ouabain (10⁻⁴ M) (Eₘₐₓ = 65.4 ± 1.9%, p > 0.05). In contrast, vasorelaxation evoked by AMQ was significantly inhibited after pre-treatments with 4-aminopyridine (10⁻³ M), tetraethylammonium (10⁻³ M) and glibenclamide (10⁻⁵ M), blockers of voltage-dependent K⁺ (Kᵥ), large and intermediate conductance Ca²⁺-activated K⁺ (BKCa) and KATP channels, respectively. Additionally, AMQ reduced CaCl₂-induced contractions in Ca²⁺-free solution containing KCl, probably due to its non-selective opening of K⁺ channels or may be acting as Ca²⁺-antagonist. Furthermore, AMQ did not interfere with Ca²⁺ release from intracellular stores mediated by either phenylephrine (10⁻⁵ M) or caffeine (0.02 M). Collectively, these results provide functional evidence that AMQ-induced hypotensive and bradycardic effects may involve the opening of K⁺ channels sensitive to 4-aminopyridine, tetraethylammonium and glibenclamide or the blockade of extra-cellular Ca²⁺ influx.

**Keywords:** Ca²⁺ influx, K⁺ channels, amodiaquine, mesenteric arteries, vasorelaxation

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sively studied. Like other quinoline containing anti-
malarials, such as CQ, quinine, quinidine and halo-
fantrine, AMQ has been reported to prolong ventric-
ular repolarization, which is evidenced by increased
QTc interval in electrocardiogram, and bradycardia
and hypotensive effects in patients with acute uncom-
pliacted *Plasmodium falciparum* at usual ther-
apeutic concentrations (12–16). Though sympto-
matic cardiac effects have not been reported after
administration of AMQ, the changes in electrocar-
diographic and hemodynamic parameters of patients
caued by AMQ warrants thorough scientific inves-
tigation. Against this background, this study was
designed to establish and provide pharmacological
basis for the vasorelaxation induced by AMQ.

**EXPERIMENTAL**

**Animals**

Male Wistar rats (280–300 g) were used for all
experiments. The animals were kept in well-venti-
lated cages at controlled temperature (21 ± 1°C) and
under controlled light cycles (12 h light/12 h dark).
Animals were maintained on normal laboratory
chow (PURINA, João Pessoa, Brazil) and tap water
*ad libitum*. The study was approved by the
Committee of Ethics for Animal Research (Comitê
De Ética Em Pesquisa Animal) CEPA No. 0805/07,
Laboratório de Tecnologia Farmacêutica,
Universidade Federal da Paraíba, João Pessoa,
Brazil.

**Drugs**

The drugs used were: L-phenylephrine
hydrochloride, acetycholine hydrochloride, Tween-
80, tetraethylammonium, glibenclamide, ouabain; 4-
aminopyridine, caffeine, ethylene glycol-bis(β-
aminoethylether)-N,N,N′,N′-tetraacetic acid EGTA)
and amodiaquine (Sigma Chemical Co., St. Louis,
MO, USA). Chemicals used for preparing Tyrode’s
solutions were: calcium chloride, glucose, magne-
sium sulfate, potassium dihydrogen phosphate, sodi-
um bicarbonate and sodium chloride (E. Merck,
Darmstadt, Germany). The stock solutions were dis-
solved in distilled water, except glibenclamide that
was dissolved in ethanol. The solutions were pre-
pared fresh on the day of experiments.

**Direct blood pressure measurements in non-anesth-
ethetized rats**

Intra-aortic blood pressure was recorded using
the technique described by Oliveira et al. (17).
Briefly, under sodium thiopental anaesthesia (45
mg/kg; *i.v*.), the lower abdominal aorta and inferior
vena cava were canulated *via* left femoral artery and
vein using polyethylene catheters. Thereafter,
catheters were filled with heparinized saline solution
and led under the skin to emerge between the scapu-
lae. Arterial pressure was measured after 24 h by
connecting the arterial catheter to a pre-calibrated
pressure transducer (Statham P23 ID; Gound,
Cleveland, OH, USA) coupled to an amplifier-
recorder (Model TBM-4M, WPI, Sarasota, FL,
USA) and then connected to a computer equipped
with an analog-digital converter board (CIO-
DAS16/JR, Computer Boards, Inc., Mansfield, MA,
USA) and CVMS software (WPI, Sarasota, FL,
USA). The data were sampled at a frequency of 500
Hz. For each cardiac cycle, the mean arterial pres-
sure (MAP) and heart rate (HR) (pulse interval)
were calculated by the computer. The venous
catheter was used for drug administration. Sodium
nitopresside (10 mg/kg; *i.v*.) was injected to check
the efficacy of the venous catheter insertion. After
cardiovascular parameters had been stabilized, the
MAP and HR values were recorded before (basel-
line) and after administration of randomized doses
of AMQ (1, 5, 10, 15 and 20 mg/kg; *i.v*.). For the
construction of a dose-response curve, the differ-
ence between the baseline and after administration
values for each dose was expressed as percentage of
baseline value. Successive injections of AMQ were
well separated by a time interval sufficient to allow
full recovery of hemodynamic parameters (40–50
minutes) (18–20).

**Preparation of rat superior mesenteric arteries
rings**

The superior mesenteric arteries from the sec-
ond order branches were quickly removed and
cleaned of adherent connective tissues and fat.
Mesenteric rings (2–4 mm length) were obtained
and suspended by cotton threads in organ bath con-
taining 10 mL of Tyrode’s solution, maintained at
37°C and gassed with a 95% O₂ + 5% CO₂ mixture
(pH 7.4). The composition of the Tyrode’s solution
(in 10⁻³ M): NaCl 158.3; KCl 4.0; CaCl₂·2H₂O 2.0;
MgCl₂·6H₂O 1.05; NaHCO₃ 10.0; NaH₂PO₄·H₂O
0.42 and glucose 5.6 (21). Rings were stabilized
with a resting tension of 0.75 g, for at least 60 min,
with constant changing of Tyrode’s solution (every
15 min) to prevent the accumulation of metabolites
that could otherwise lead to misinterpretation of results (22). The isometric tension was recorded by a
force-displacement transducer (Miobath-4, WPI,
Sarasota, FL, USA) coupled to an amplifier-recorder
(Transbridge-4; WPI, Sarasota, FL, USA) and a
computer equipped with an analog–to–digital con-
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In some experiments, the endothelium layer of the rings was removed by gently rubbing the external surface with a finger moistened with Tyrode’s solution. Endothelial integrity was assessed qualitatively by the degree of relaxation caused by acetylcholine (10⁻⁵ M) in the presence of contractile tone induced by phenylephrine (PHE) (10⁻⁵ M). Rings were considered to be endothelium-denuded when acetylcholine-induced relaxant effects were less than 10% and, endothelium-intact when the relaxant effects were more than 90%.

Effect of AMQ on contractions induced by phenylephrine (10⁻⁵ M) or KCl (0.08 M) in isolated rat superior mesenteric arteries

After equilibration, steady tension was evoked by phenylephrine (10⁻⁵ M) for endothelium-intact and -denuded rings to induce contraction of similar magnitude and AMQ was added cumulatively (10⁻⁸ ñ 10⁻³ M). The ability of AMQ to attenuate KCl (0.08 M)-induced sustained contraction in the rings was also examined for both endothelium-intact and -denuded rings. The relaxations were measured by comparing the developed tension before and after addition of AMQ.

Investigation of the role of K⁺ channels in AMQ-induced vasorelaxation in isolated superior mesenteric arteries

In another set of experiments, the rings without endothelium were pre-contracted with phenylephrine (10⁻⁵ M) for 30 min after being pre-incubated with one of the following inhibitors: glibenclamide (10⁻⁵ M), selective blocker of K_ATP channels, tetraethylammonium (TEA) (10⁻³ M), non-selective blockers of BKᵦ channels, 4-aminopyridine (4-AP) (10⁻³ M), a selective blocker of Kᵥ channels and ouabain (10⁻⁴ M), a selective inhibitor of Na⁺⁻K⁺ ATPase. The concentration used for each inhibitor of K⁺ channels is sufficient to antagonize selectively those channels in arterial smooth muscle (19, 23–25). After stabilization of the tonic contraction induced by phenylephrine (10⁻⁵ M), increasing cumulative concentrations of AMQ (10⁻⁸ ñ 10⁻³ M) were added to the organ bath.

Effect of AMQ on contractions induced by CaCl₂, and Ca²⁺ release from intracellular stores

To further investigate the mechanism of vasorelaxation induced by AMQ, concentration-response curves to CaCl₂ were constructed using endothelium-denuded rings (24). Briefly, the rings were pre-contracted with KCl (0.06 M) to confirm tissue viability. The Tyrode’s solution was replaced with depolarizing Tyrode’s solution (KCl 0.06 M) nominally without Ca²⁺ (15 min). Thereafter, concentration-response curves to CaCl₂ (10⁻⁶ M ñ 10⁻² M) were constructed in the absence or presence of AMQ (10⁻⁶, 10⁻⁵, 10⁻⁴, 10⁻³ M). To determine the effect of AMQ on the release of Ca²⁺ from intracellular stores, the endothelium-denuded rings were pre-contracted with KCl (0.06 M), washed and exposed to Ca²⁺-free Tyrode’s solution containing EGTA (10⁻³ M). The rings were then stimulated with phenylephrine (10⁻⁵ M) or caffeine (0.02 M) (26). The contractions of agonists were obtained in absence (control) or after incubation with AMQ (10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵ and 10⁻⁴ M).

Statistical analysis

Two pharmacological parameters were ana-
lyzed in this study; $E_{\text{max}}$ (maximal effect generated by agonist) and $pD_2$ ($-\log IC_{50}$). Values are expressed as the means ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) or Student’s t-test using GraphPad Prism TM 5.0 version software, San Diego, CA, USA. Post hoc comparisons were performed using Dunnett’s test. The level of significance considered in all the tests was 0.05.

RESULTS

Effect of AMQ on mean arterial pressure (MAP) and heart rate (HR) in non-anesthetized rats

In this study, the baseline values of MAP and HR were 118 ± 2 mmHg and 334 ± 6 bpm, respectively. AMQ at doses 10, 15 and 20 mg/kg (i.v.) induced hypotension by 22.3 ± 1.2%, 34.5 ± 3.1% and 32.1 ± 3.4%, respectively (expressed as percentage of baseline values) (Fig. 2). The hypotensive response was found to be short-live (4 ± 1 min). The hypotensive response was accompanied by prolonged and dose-dependent bradycardia (4.1 ± 1.6%, 8.4 ± 2.3%, 17.0 ± 3.3%, 39.3 ± 3.5% and 53.2 ± 2.9% at doses of 1, 5, 10, 15 and 20 mg/kg, respectively). The bradycardic effect lasted for 10 ± 3 min at each dose.

Effect of AMQ on mesenteric rings pre-contracted with phenylephrine or KCl

AMQ at $10^{-6} - 10^{-3}$ M significantly ($p < 0.05$) relaxed the sustained contractions induced by phenylephrine (PHE) and KCl in a concentration-dependent manner in both endothelium-intact and -denuded rings (Fig. 3). The $E_{\text{max}}$ and $pD_2$ values of AMQ for intact and denuded rings in both agonists are statistically similar. Thus, endothelium removal has no effect on the vasorelaxation induced by AMQ. AMQ was active in inhibiting both the PHE- and KCl-induced contractions of the arterial rings. The $IC_{50}$ values of AMQ were $1.34 ± 0.04 \times 10^{-5}$ M and $5.88 ± 0.05 \times 10^{-5}$ M for the inhibition of PHE- and KCl-induced contractions of the rings, respectively.

Role of K+ channels in AMQ-induced vasorelaxation in superior mesenteric arteries

In denuded rings, the incubation with KCl (0.02 M) before constriction with PHE significantly decreased the $pD_2$ values of AMQ when compared to controls (Table 1), indicating the involvement of K+ channels in the vasorelaxation induced by AMQ. Similarly, incubation of denuded rings with glibenclamide ($10^{-5}$ M), tetraethylammonium ($10^{-3}$ M) and 4-aminopyridine ($10^{-3}$ M) shifted the concentration-response curve for AMQ to right with reduction of the $pD_2$ values (Table 1 and Fig. 4). Interestingly, in endothelium-denuded rings, the concentration-response curve for AMQ was insignificantly changed after incubation with ouabain ($10^{-4}$ M), (Fig. 4B).
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Effect of AMQ on contractions induced by CaCl2, and Ca2+ release from intracellular stores

Pre-incubation of endothelium denuded rings with AMQ attenuated CaCl2-induced contraction in Ca2+-free medium containing KCl (0.06 M). Administration of CaCl2 induced a concentration-dependent contraction of mesenteric rings ($E_{\text{max}}$ = 100%; $pD_2$ = 3.49 ± 0.06, Table 2). Pre-incubation of the rings with AMQ at 10⁻⁴ M and 10⁻³ M significantly ($p < 0.05$) inhibited the CaCl2-induced contraction (Fig. 5). The effects of AMQ on the $E_{\text{max}}$ and $pD_2$ values for CaCl2-induced contractions are given in Table 2. In contrast, AMQ did not produce any remarkable effect on the transient contractions induced by phenylephrine (10⁻⁵ M) and caffeine (0.02 M) in endothelium denuded rings in Ca²⁺-free media containing EGTA (10⁻³ M) (Fig. 6).

DISCUSSION AND CONCLUSION

The present study was designed to clarify the pharmacological basis for the observed vasorelaxation induced by AMQ in rat superior mesenteric arteries. The choice of this artery is due to its substantial contribution to the regulation of systemic circulation and, also reflects the variation of vascular resistance in circulation (27, 28). The major finding of this work was that AMQ induced concentration-dependent relaxation of rat mesenteric arteries that may be mediated by the blockade of Ca²⁺ influx and the opening of K⁺ channels sensitive to 4-aminopyridine, tetraethylammonium and glibenclamide with a resultant membrane hyperpolarization/repolarization. Furthermore, in this study AMQ induced hypotensive and bradycardic effects in non-anesthetized rats. The hypotensive and bradycardic effects of AMQ may be due, at least in part, to the blockade of Ca²⁺ influx by AMQ. The effects are common with cholinergic compounds, which are known to cause a fall in blood pressure by activation

<table>
<thead>
<tr>
<th>Groups</th>
<th>$E_{\text{max}}$ (%)</th>
<th>$pD_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>66.9 ± 0.30</td>
<td>4.87 ± 0.03</td>
</tr>
<tr>
<td>KCl (0.02 M)</td>
<td>65.8 ± 1.33</td>
<td>3.83 ± 0.04*</td>
</tr>
<tr>
<td>TEA (10⁻³ M)</td>
<td>65.0 ± 1.60</td>
<td>3.71 ± 0.06*</td>
</tr>
<tr>
<td>Glibenclamide (10⁻³ M)</td>
<td>65.6 ± 1.13</td>
<td>3.82 ± 0.05*</td>
</tr>
<tr>
<td>4-AP (10⁻³ M)</td>
<td>65.4 ± 1.64</td>
<td>3.92 ± 0.03*</td>
</tr>
<tr>
<td>Ouabain (10⁻³ M)</td>
<td>66.4 ± 1.92</td>
<td>4.84 ± 0.05</td>
</tr>
</tbody>
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Values are the mean ± SEM, n = 7–8 experiments. * Significantly different from control ($p < 0.05$) (ANOVA followed by Dunnett’s multiple comparison test).

Figure 4. Relaxation responses induced by amodiaquine on endothelium-denuded rat mesenteric rings pre-contracted with phenylephrine in the presence of 4-aminopyridine (4-AP) (10⁻³ M), glibenclamide (10⁻³ M) (A) and ouabain (10⁻³ M), tetraethylammonium (TEA) (10⁻³ M) (B). The rings were pre-incubated with the inhibitors for 30 min. Steady tension was evoked by phenylephrine (10⁻⁵ M) and amodiaquine (10⁻⁷ – 10⁻³ M) was added cumulatively.
Furthermore, AMQ was found to be effective in relaxing the endothelium-denuded rings preconstricted with both phenylephrine and KCl. Precisely, the IC50 of AMQ for the inhibition on phenylephrine- and KCl-induced contractions were 1.34 ± 0.04 × 10^-5 M and 5.88 ± 0.05 × 10^-5 M, respectively. It is well known that KCl induces smooth muscle contraction through the activation of voltage-dependent calcium channels and subsequent release of calcium from sarcoplasmic reticulum (30, 31) whereas phenylephrine-induced vasoconstriction is mediated by the stimulation of G-proteins coupled to α-adrenoceptors (32). Thus, both contractile agents produce a significant increase in intracellular calcium concentration through calcium of muscarinic receptors located on the epithelium of blood vessels (29). Furthermore, there may be need to perform experiments to confirm the involvement of muscarinic receptors in the bradycardic effect shown by AMQ. In agreement with this study, Ngouesse et al. (12) observed bradycardia in 16 of 20 AMQ-treated patients on day 2 of administration that corresponds to the time when maximal cumulative plasma concentration of AMQ was reached.

In the present study, AMQ concentration-dependently reduced the contractions induced by phenylephrine or KCl in endothelium-intact and -denuded arterial rings with statistically similar E_max and pD_2 values, indicating that endothelium is not required for the relaxation induced by this anti-malarial. Furthermore, AMQ was found to be effective in relaxing the endothelium-denuded rings preconstricted with both phenylephrine and KCl. Precisely, the IC50 of AMQ for the inhibition on phenylephrine- and KCl-induced contractions were 1.34 ± 0.04 × 10^-5 M and 5.88 ± 0.05 × 10^-5 M, respectively. It is well known that KCl induces smooth muscle contraction through the activation of voltage-dependent calcium channels and subsequent release of calcium from sarcoplasmic reticulum (30, 31) whereas phenylephrine-induced vasoconstriction is mediated by the stimulation of G-proteins coupled to α-adrenoceptors (32). Thus, both contractile agents produce a significant increase in intracellular calcium concentration through calcium...
entry. Therefore, it can be suggested that the vasorelaxant effects observed for AMQ may be due to an endothelium-independent mechanism that could be linked to the blockade of calcium influx into the vascular smooth muscle cells (VSMCs).

Another aspect investigated in this study was whether AMQ-induced vasorelaxation could be related to inhibition of Ca$^{2+}$ influx from extracellular medium. It was observed that AMQ at $10^{-4}$ M and $10^{-3}$ M decreased CaCl$_2$-induced contraction in Ca$^{2+}$-free medium containing KCl. Taken together, these results support the notion that AMQ can block Ca$^{2+}$ influx through Ca$^{2+}$ channels on VSMCs. This observation does not rule out the possibility that AMQ reduces the sensitivity of the contractile filaments to Ca$^{2+}$. This led to the investigation on whether AMQ could exert its vasorelaxant effects by interfering with the release of intracellular calcium, [Ca$^{2+}$], via the phosphoinositide-dependent or -independent pathway following receptors activation. In Ca$^{2+}$-free media containing EGTA, AMQ did not interfere with the transient contractions induced by either phenylephrine or caffeine. It seems likely that the vascular effect of AMQ does not involve the reduction in Ca$^{2+}$ release from intracellular stores, there may be need to confirm this observation using inhibitors of intracellular Ca$^{2+}$-release.

The opening of K$^+$ channels in the cell membrane of smooth muscle cells in arteries increases K$^+$ efflux causing membrane potential hyperpolarization, which leads to vasodilatation (23). Tetraethylammonium (TEA) ($10^{-3}$ M), a non-selective blocker of large and intermediate conductance Ca$^{2+}$-activated K$^+$ channels (BK$_{Ca}$), produced a displacement of the concentration-response curve for AMQ to the right, probably indicating the participation of K$^+$ channels in the vasorelaxation action induced by this antimalarial. Vascular smooth muscle cells are known to express different types of K$^+$ channels (33). Agents that block these channels are useful tools for exploring the role of a particular K$^+$ channel. In the present study, both glibenclamide ($10^{-4}$ M) and 4-aminopyridine ($10^{-4}$ M) produced rightward displacement of the concentration-response curves for AMQ, suggesting that K$^+$ channels that are sensitive to glibenclamide and 4-aminopyridine may be involved in the vasorelaxation induced by AMQ. In contrast, ouabain ($10^{-4}$ M), an inhibitor of Na$^+$-K$^+$ ATPase (19, 34) did not affect the concentration-response curves for AMQ. Therefore, Na$^+$-K$^+$ ATPase may not be involved in the vasorelaxant effect elicited by AMQ.

In conclusion, the vasorelaxation-induced by AMQ occurred through an endothelium-inde-pendent mechanism that may involve the blockade of extracellular Ca$^{2+}$ influx by interfering with voltage-operated channels. In addition, the relaxant effect of AMQ may be linked to K$^+$ channels that are sensitive to 4-aminopyridine, tetraethylammonium and glibenclamide. However, further studies are required on the cardiac evaluation of different types of antimalarials in order to ensure their safety.

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REFERENCES


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