The Effect of Chloroquine on Renal Function and Vasopressin Secretion: A Nitric Oxide-Dependent Effect

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ABSTRACT
We have previously reported that chloroquine administration increases plasma vasopressin concentration and urinary sodium excretion in Sprague-Dawley rats. Because chloroquine has also been shown to stimulate nitric oxide production, the aim of this study was to determine whether nitric oxide mediates chloroquine-induced changes in renal function and secretion of vasopressin. Sprague-Dawley rats (n = 6–8/group) were infused with 2.5% dextrose under Intraval anesthesia (100 mg kg\(^{-1}\) i.p.). After 3-h equilibration and a control hour, animals received either vehicle, chloroquine (0.04 mg h\(^{-1}\)), \(N^2\)-nitro-L-arginine methyl ester (\(L\)-NAME) (nitric-oxide synthase inhibitor, 60 \(\mu\)g kg\(^{-1}\) h\(^{-1}\)), or combined chloroquine and \(L\)-NAME over the next hour. \(L\)-NAME or vehicle infusion continued for a further recovery hour. Plasma was collected from a parallel group of animals for vasopressin radioimmunoassay. Chloroquine stimulated a significant increase (\(p < 0.05\)) in urine flow rate, glomerular filtration rate, and sodium excretion over the hour of infusion, in comparison with vehicle-infused rats. These effects continued after cessation of chloroquine, reaching maxima in the following recovery hour. Coadministration of \(L\)-NAME abolished these effects, returning all parameters to levels comparable with those in vehicle-infused animals. Chloroquine administration was accompanied by a significant increase (\(p < 0.05\)) in plasma vasopressin, which was also reversed by \(L\)-NAME. The effects of chloroquine on renal function and vasopressin secretion seem to be mediated by pathways involving nitric oxide. These data suggest that chloroquine may stimulate nitric-oxide synthase both centrally, stimulating vasopressin secretion, and within the kidney, where it modulates glomerular hemodynamics and tubular function.

Chloroquine was first prepared by Andersag and colleagues in the Bayer group in 1934 (Andersag, 1934) and is still one of the most widely used antimalarial drugs (Sharma and Mishra, 1999). It is also used clinically to treat rheumatoid arthritis and systemic lupus erythematosus (Ducharme and Farinotti, 1996). However, increasing evidence suggests that chloroquine may also influence renal function with potentially important consequences for patients whose fluid status is challenged.

We have previously reported that acute chloroquine administration in the anesthetized rat increased renal Na\(^{+}\) excretion and plasma arginine vasopressin concentration (Musabayane et al., 1994, 1996). The natriuretic effect seemed to be mediated by vasopressin, via \(V_1\) receptors (Musabayane et al., 1997), because chloroquine administration to Brattleboro, vasopressin-deficient rats had no effect on Na\(^{+}\) excretion (Musabayane et al., 1996). This chloroquine-induced natriuresis was not associated with a change in blood pressure or glomerular filtration rate (Musabayane et al., 1994) nor, surprisingly, urine flow rate, despite the concurrent increase in plasma vasopressin concentration. One explanation for these apparently contradictory effects is provided by the observation that chloroquine, at \(10^{-6}\) M, significantly suppressed a vasopressin-stimulated increase in cAMP production in isolated inner medullary collecting ducts, suggesting that chloroquine may interfere with the normal antidiuretic response to vasopressin by reducing cAMP formation (Musabayane et al., 2000b).

One potential mediator of this inhibitory effect is nitric oxide, which has been shown to inhibit vasopressin-stimulated cAMP generation (Wang et al., 1999). Furthermore, nitric oxide has a marked influence on renal function, increasing glomerular filtration rate (GFR) (Klahr, 1999) and affecting sodium transport and excretion (Roczniak and Burns, 1996; Eitle et al., 1998). Chloroquine has been shown to stimulate nitric-oxide synthesis in murine, porcine, and human endothelial cells (Ghigo et al., 1998) and has been shown to induce venodilation in human hand veins through a dose-dependent, nitric oxide-mediated mechanism (Abiose et al., 1997). The mechanism by which chloroquine stimulates nitric oxide is not yet known; however, it has been suggested

ABBREVIATIONS: GFR, glomerular filtration rate; \(L\)-NAME, \(N^2\)-nitro-L-arginine methyl ester; ANOVA, analysis of variance; SNK, Student-Newman-Keuls; NOS, nitric-oxide synthase.
that this action is dependent on its weak base properties and limitation of the availability of iron (Ghigo et al., 1998).

Accordingly, the aim of this study was to determine the potential role of nitric oxide in mediating chloroquine's influence on renal function and vasopressin secretion. To identify potentially subtle changes in renal function we have used a novel, servo-controlled fluid replacement system to match intravenous infusion rate to urinary excretion, thereby maintaining an euvoalaemic state. This has an advantage over previous studies (Musabayane et al., 1993, 1996) that used a constant infusion protocol that leads to extracellular volume expansion and compensatory changes in renal function that may mask some of the effects of chloroquine.

**Materials and Methods**

All experiments were performed under the authority of a UK Home Office Project License and received local ethical approval.

**Animal Preparation.** Male Sprague-Dawley rats were purchased from Charles River UK Limited (Margate, Kent, UK) and were held in the School of Biological Sciences where they had free access to food (Beekay Rat and Mouse Standard Diet; Bantin and Kingman Ltd., Hull, UK) and water, with a 12-h light and 12-h dark cycle before experimentation. The weight of animals at renal function study was between 330 and 340 g. Animals were anesthetized with Intraval (100 mg kg⁻¹ body weight, thiopentone sodium BP, Rhône-Poulenc Rorer Limited, Nenagh, Ireland) and transferred to a hot-plate that maintained body temperature, monitored by a rectal probe, at 37°C throughout the experiment. Cannulae were inserted into an external jugular vein, carotid artery, and the bladder, and a tracheotomy was performed as described previously (Ashton and Balment, 1988). Animals remained under anesthesia for the duration of the experiment, receiving a supplemental dose of Intraval (10 mg kg⁻¹ body weight) as necessary.

**Servo-Controlled Fluid Replacement System.** This system relies on urine flow information from a balance being transmitted to an adjustable pump via a computer. A program developed at the University of Manchester (Burgess et al., 1993) allows the computer to detect changes in urine output gravimetrically and make changes to the infusion rate of the pump accordingly, to precisely replace intravenously the volume of fluid lost as urine.

The rat was positioned so that all urine produced flowed directly from the bladder catheter into a preweighed plastic vial placed on an electronic balance (model L2200 P; Sartorious, Gottingen, Germany), from the bladder catheter into a preweighed plastic vial placed on an electronic balance (model L2200 P; Sartorious, Gottingen, Germany), and transferred to a hot-plate that maintained body temperature, monitored by a rectal probe, at 37°C throughout the experiment. Cannulae were inserted into an external jugular vein, carotid artery, and the bladder, and a tracheotomy was performed as described previously (Ashton and Balment, 1988). Animals remained under anesthesia for the duration of the experiment, receiving a supplemental dose of Intraval (10 mg kg⁻¹ body weight) as necessary.

**Urine Flow Rate.** Urine flow rate throughout the 3 h postequilibration period is shown in Fig. 1. Repeated measures ANOVA revealed significant differences both over time ($F_{3,62} = 29.3, p < 0.001$) and between drug treatments ($F_{3,21} = 9.2, p < 0.001$). Before the infusion of chloroquine ± L-NAME, urine flow rate was comparable in all groups of animals. Upon chloroquine administration, urine flow increased significantly, compared with control rats (post hoc Student-Newman-Keuls test control versus chloroquine, $p < 0.05$), within 20 min of the start of the infusion. This increase in urine output continued into the recovery hour after the chloroquine treatment ceased. By the end of the experiment, all rats then received 2.5% dextrose replacement for a 1-h control period, after which vehicle animals continued to receive 2.5% dextrose for the remaining 2 h of the experiment. In the chloroquine-treated group, infusion of chloroquine (0.04 mg h⁻¹ chloroquine diphosphate (Sigma-Aldrich, Poole, Dorset, UK), previously shown in our hands to affect renal function in the anesthetized rat; Musabayane et al., 1993) was started via the constant infusion pump for 1 h, after which the infusate was switched to 2.5% dextrose for the final hour of the experiment.

In the L-NAME-treated group, L-NAME (60 μg kg⁻¹ h⁻¹ (Sigma-Aldrich), previously shown to be effective in our hands at this dose in inhibiting nitric-oxide synthase in the anesthetized rat with no alteration in blood pressure; Gouldsborough and Ashton, 2001) was infused for 2 h after the control period. In the final group, combined L-NAME and chloroquine infusion began after the 1-h control period. Chloroquine infusion ceased after 1 h and rats continued to receive L-NAME for the final hour. Urine samples were collected every 10 min after the equilibration period, and blood samples were collected at 0.5, 1.5, and 2.5 h postequilibration. The blood samples (0.6 ml) were collected from the carotid artery and a similar volume of dextrose solution was replaced. Plasma was separated by centrifugation and stored at 4°C before analysis.

Parallel groups of animals ($n = 6$ group) equivalent to those used for renal studies were prepared specifically to collect blood for arginine vasopressin radioimmunooassay. Blood samples were taken from animals undergoing servo-controlled fluid replacement midway through the drug (chloroquine, L-NAME, or in combination) treatment hour. Animals were decapitated and trunk blood (5–7 ml) was collected into tubes held on ice containing 100 μl of 0.125 mol of EDTA (Sigma-Aldrich) and 250 μl of ammonium heparin (BDH, Poole, Dorset, UK). Plasma was separated after centrifugation for 10 min and stored at −20°C before measurement of plasma vasopressin concentration by radioimmunoassay as described previously (Warne et al., 1994). The assay sensitivity was 1.2 fmol ml⁻¹; coefficients of variation were determined using a pool of plasma with a measured vasopressin concentration of 4 pg ml⁻¹, interassay variation was 8.2 ± 0.8% ($n = 5$) and intra-assay variation was 11.4 ± 1.5% ($n = 10$).

**Analysis.** Osmolality was determined in urine samples (freezing point depression, LH Roebling osmometer; LH Roebling, Berlin, Germany) and the concentration of sodium was measured in both plasma and urine (flame photometry, Corning 480; Corning Ltd, Halstead, Essex, UK). [3H]Inulin was determined in plasma and urine using a 1900CA Tri-Carb liquid scintillation analyzer (Canberra Industries, Meriden, CT) beta counter.

**Statistical Analysis.** Data are presented as the mean ± S.E.M. Statistical analysis was performed using SPSS for Windows (standard version; SPSS, UK Ltd., Surrey, UK). Comparisons between groups over time were by repeated measures ANOVA and comparisons within control, treatment, or recovery periods were by ANOVA followed by Student-Newman-Keuls test. Significance was ascribed at the 5% level.
urine output was 3.5 times higher in the chloroquine-treated 
rats compared with the vehicle group.

l-NAME was administered to a separate group of rats for 
2 h. During the 1st h, urine flow rate fell compared with 
vehicle rats ($p < 0.05$) but began to increase in the 2nd h such 
that urine output was greater than in vehicle-infused rats for 
the last 20 min of the experiment ($p < 0.05$). In marked 
contrast to the chloroquine alone group, urine flow in rats 
receiving both chloroquine and l-NAME was significantly 
lower than the vehicle group flow rate ($p < 0.05$) over the last 
90 min of the experiment by almost 50%.

There were no differences between groups for all renal 
parameters during the initial postequilibration hour (Figs. 
2–4; ANOVA 1st hour, GFR $F_{3,22} = 1.4$, $p = 0.263$; Na$^+$ 
excretion $F_{3,22} = 0.684$, $p = 0.572$; urine osmolality $F_{3,22} = 
0.508$, $p = 0.681$). For ease of comparison, in subsequent 
graphs the mean values are presented for the control, post-

equilibration hour (1st h), the hour of drug treatment (2nd h), 
and the recovery hour (3rd h).

GFR. During the hour of chloroquine infusion, a significant 
increase in the GFR was seen by comparison with vehicle 
rats (ANOVA 2nd h, $F_{3,22} = 15.22$, $p < 0.001$; post hoc 
SNK test vehicle versus chloroquine, $p < 0.05$) (Fig. 2), which 
continued into the recovery hour (ANOVA 3rd h, $F_{3,22} = 
29.07$, $p < 0.001$; post hoc SNK test vehicle versus chloro-
quine, $p < 0.05$) after chloroquine administration ceased, 
reaching a maximum of $7.8 \pm 0.9$ ml min$^{-1}$ at 150 min. 
Coadministration of l-NAME with chloroquine completely 
abolished this effect on GFR ($p < 0.05$). In these rats, GFR 
did not differ significantly from the vehicle rats. l-NAME 
alone did not induce a significant change in GFR in compar-
ison with vehicle rats.

Sodium Excretion. During chloroquine treatment, there 
a was significant increase in Na$^+$ excretion (ANOVA 2nd h, 
$F_{3,22} = 9.45$, $p < 0.001$; post hoc SNK test vehicle versus 
chloroquine, $p < 0.001$) in comparison with vehicle-infused 
animals (Fig. 3), which reached a maximum of $145 \pm 21$ µmol 
min$^{-1}$ at 120 min from starting chloroquine infusion. During 
the recovery hour Na$^+$ excretion remained elevated (ANOVA 
3rd h, $F_{3,22} = 23.09$, $p < 0.001$; post hoc SNK test vehicle 
versus chloroquine $p < 0.001$), compared with vehicle-treated 
rats, at a rate almost double that observed during the hour of 
chloroquine treatment with a maximum excretion rate of 
$265 \pm 47$ µmol min$^{-1}$ at 180 min. Coadministration of l-
NAME with chloroquine reduced sodium excretion to a sig-
nificant degree in both hours in comparison with rats receiv-
ing chloroquine alone ($p < 0.05$), but these remained above 
levels displayed by vehicle-infused rats ($p < 0.05$). l-NAME 
administration alone did not induce any alteration in sodium 

excretion during the 2 h of L-NAME infusion in comparison with the vehicle group.

**Urine Osmolality.** Urine osmolality, a measure of urine-concentrating ability, is shown in Fig. 4. During the hour of chloroquine infusion urine osmolality was significantly reduced (ANOVA 2nd h, $F_{3,22} = 5.54$, $p = 0.005$; post hoc SNK test vehicle versus chloroquine, $p < 0.05$) in comparison with vehicle-infused animals, reaching its lowest level of 176 ± 21 mOsm kg H$_2$O$^{-1}$ at 120 min. In the recovery hour, there was a further reduction in urine osmolality (ANOVA 3rd h, $F_{3,22} = 2.94$, $p = 0.05$; post hoc SNK test vehicle versus chloroquine, $p < 0.001$) to a low of $106 \pm 16$ mOsm kg H$_2$O$^{-1}$ at 180 min, which was associated with the continued rise in urine flow rate (Fig. 1). L-NAME coadministration with chloroquine restored urine osmolality to levels seen in vehicle-infused animals. There was a modest but significant ($p < 0.05$) increase in osmolality during the 1st h of L-NAME infusion alone in comparison with the vehicle group, but this returned to baseline in the 2nd h.

**Effect of Chloroquine/L-NAME Administration on Plasma Vasopressin.** Plasma vasopressin concentrations after 30 min of drug administration in rats treated with chloroquine in the presence or absence of L-NAME are shown in Fig. 5. Chloroquine treatment induced a marked increase in plasma vasopressin concentration in comparison with vehicle rats (ANOVA, $F_{3,20} = 25.66$, $p < 0.001$; post hoc Student-Newman-Keuls test vehicle versus chloroquine, $p < 0.05$). L-NAME alone significantly reduced the plasma vasopressin concentration of rats compared with the vehicle group ($p < 0.05$). Similarly, administration of L-NAME with chloroquine abolished the stimulatory effect of chloroquine on plasma vasopressin ($p < 0.05$).

These changes in renal function and vasopressin secretion were not associated with any change in mean arterial blood pressure over the experimental period (vehicle, $123 \pm 3$; chloroquine, $122 \pm 2$; L-NAME, $123 \pm 4$; and chloroquine/L-NAME, $120 \pm 7$ mm Hg).

**Discussion**

The results of the current study confirm our previous observations that chloroquine increases renal Na$^+$ excretion in anesthetized rats (Musabayane et al., 1993, 1996, 2000a), but contrast with our previous report that acute chloroquine administration has no effect on urine flow rate or GFR (Musabayane et al., 1996). In the present study, where careful attempts have been made to secure fluid balance in anesthetized rats, there was a significant increase in both urine flow rate and GFR that may be explained, at least in part, by the different protocols used in the two studies. The previous work used a continuous infusion protocol that inevitably leads to expansion of extracellular fluid volume, whereas the servo-controlled fluid replacement system used here avoids this complication, which may mask drug effects. Therefore, the glomerular effects of chloroquine reported here are likely to be more representative of the renal action of chloroquine in the absence of compensatory responses after extracellular fluid volume expansion. Such an increase in GFR is also likely to favor a diuretic response, as observed in the current study.

Evidence from the present study and previous reports of the stimulatory effects of chloroquine on nitric oxide generation suggest that these effects on GFR may be mediated by nitric oxide. L-NAME alone induced a modest fall in GFR and urine flow rate, in accord with the known action of nitric oxide on vascular tone in the glomerulus. Under control conditions, nitric oxide acts to counterbalance the vasoconstrictor influence of angiotensin II on the afferent arterioles (Kone and Baylis, 1997), thereby lowering preglomerular resistance and increasing GFR. Hence, nitric-oxide synthase inhibition results in a fall in GFR (Granger et al., 1992; Gouldsborough and Ashton, 2001) in the absence of a change in systemic blood pressure, as was the case in the current study. Nitric oxide has also been shown to inhibit proximal
tubule fluid reabsorption (Eitle et al., 1998); hence, blockade of this effect, coupled with the fall in GFR, could also account for the modest fall in urine flow rate during the 1st h of L-NAME infusion. Over the 2nd h of L-NAME infusion urine flow began to increase, such that urine flow rate in L-NAME-treated rats was significantly higher than that of vehicle-infused rats. This is consistent with the inhibition of vasopressin by L-NAME compared with the somewhat elevated vasopressin levels of the vehicle group. Although these effects will have contributed to the response seen upon combined L-NAME and chloroquine administration, it is unlikely that the large changes seen in GFR, urine flow, and sodium excretion were due to inhibition of basal nitric oxide production alone. L-NAME administration in this study reduced GFR by 55% to baseline levels during chloroquine treatment, which opens up the possibility that chloroquine may have increased GFR by increasing nitric oxide synthesis within the glomerulus.

Chloroquine also had profound effects on electrolyte excretion. In the current study, there was a 242% increase in renal Na⁺ excretion during the hour of chloroquine treatment and a 433% increase in the subsequent hour. Although the influence of nitric oxide cannot be discounted because nitric oxide inhibits sodium and water reabsorption in the proximal convoluted tubule (Eitle et al., 1998), this is also likely to reflect the increase in GFR and thus filtered load of Na⁺. Chloroquine infusion resulted in an increase in the filtered load of Na⁺ of 124% in comparison with vehicle-infused animals and was associated with a Na⁺ fractional excretion of 2% compared with 1% in the vehicle group, which suggests additional chloroquine effects on renal tubular handling of sodium.

We have previously reported that vasopressin has a natriuretic action at physiological concentrations (Balment et al., 1984), which can be inhibited by V₁ receptor antagonism (Musabayane et al., 1997). Acute chloroquine administration under conditions of volume expansion and euvoamaemia increased plasma vasopressin concentrations, as well as increasing Na⁺ excretion (Musabayane et al., 1996). Furthermore, the chloroquine-induced increase in Na⁺ excretion was inhibited by V₁ receptor antagonism, albeit in volume-expanded rats (Musabayane et al., 1996). Critically, chloroquine failed to increase Na⁺ excretion in vasopressin-deficient Brattleboro rats (Musabayane et al., 1996), which provides strong support for our assertion that the natriuresis observed in the current study was due, in large part, to a chloroquine-mediated increase in plasma vasopressin. This is further supported by the results of quantitative reverse transcription-polymerase chain reaction studies that have demonstrated the presence of V₁ receptors in the ascending limb (Terada et al., 1993; Imbert-Teboul and Champigneulle, 1995), an important site of Na⁺ reabsorption.

Clearly, chloroquine may be operating through a number of different mechanisms that lead to an increase in sodium excretion. However, despite the marked natriuresis observed, the plasma sodium concentration was not different from that of vehicle-infused animals (vehicle 135 ± 3 versus chloroquine 134 ± 3 mM).

Chloroquine has been reported to reach its maximum plasma concentration 60 to 90 min after intravenous, intramuscular, or oral administration (Salako et al., 1987). Urine flow rate in the present study started to increase 20 min after the start of chloroquine administration and continued to increase after cessation of chloroquine infusion, reflecting the long half-life of chloroquine. This diuretic effect seems to be contradictory to the observed marked increase in plasma vasopressin, but may perhaps be explained by the actions of nitric oxide on vasopressin-mediated water reabsorption.

Garcia et al. (1996) demonstrated that nitric oxide decreased vasopressin-stimulated water and sodium transport in isolated cortical collecting ducts by a mechanism involving cGMP-mediated inhibition of cAMP (Wang et al., 1999). Nitric oxide has been shown to buffer the action of vasopressin in the inner medullary collecting duct (Park et al., 1998), which is in accord with our previous observation that chloroquine reduced vasopressin-stimulated cAMP in rat collecting ducts (Musabayane et al., 2000b). The medullary collecting duct, vasopressin's major target, is the segment with greatest nitric-oxide synthase enzymatic activity, expressing mRNA for neuronal NOS, inducible NOS, and endothelial NOS (Wu et al., 1999) and hence may be a target site for chloroquine stimulation of nitric oxide, rendering the collecting duct unresponsive to the action of secreted vasopressin. The observed diuresis is thus apparently a combination of several effects of chloroquine, including increased GFR and solute excretion (osmotic diuresis) and reduced action of vasopressin, each of which may involve nitric oxide.

Although nitric oxide inhibits the actions of vasopressin on the nephron, it seems to have a stimulatory effect on vasopressin release. Nitric-oxide synthase has been shown to be present and colocalized with vasopressin in the magnocellular neurons of the supraoptic and paraventricular nuclei, as well as in the posterior pituitary gland (Calka and Block, 1993). Furthermore, nitric-oxide synthase activity increases in the posterior pituitary during salt loading and in the supraoptic nuclei during dehydration (Goyer et al., 1994). Intracerebroventricular (i.c.v.) administration of L-arginine leads to a significant increase in vasopressin secretion, whereas i.c.v. L-NAME injection blocked the effect (Eriksson et al., 1982; Cao et al., 1996). These observations suggest that nitric oxide may participate in the regulation of vasopressin release. In the present study, chloroquine administration resulted in a marked increase in plasma vasopressin concentration that was completely blocked by L-NAME administration. This suggests that the chloroquine-induced increase in vasopressin secretion observed in this study was mediated largely through a nitric oxide-dependent mechanism. Interestingly, the application of L-NAME alone also reduced plasma vasopressin levels to below those of the vehicle-infused group, which were somewhat elevated by comparison with conscious, untreated animals (Windle et al., 1993) in association with the acute surgery and anesthesia.

In conclusion, this study has shown that acute chloroquine administration to euvolaemic rats results in an increase in GFR, urine flow rate, and sodium excretion as well as a marked increase in plasma vasopressin. The renal effects of chloroquine that were dependent, at least in part, upon stimulated vasopressin secretion were inhibited by L-NAME, supporting a role for nitric oxide in chloroquine-induced secretion of vasopressin. Chloroquine may also exert direct actions on the kidney, at the level of either the glomerulus or the tubule, which are independent of vasopressin. All of the renal actions of chloroquine reported in this study were blocked by L-NAME administration, suggesting that nitric oxide is likely
to be involved in mediating both the vasopressin-dependent and independent components of chloroquine’s effects.

References
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