(+)-Norfenfluramine injection into the nucleus accumbens also reduced the effect of muscimol, but had no effect on food intake of starved rats. This suggests that the effect of systemically injected (+)-fenfluramine (Borsini et al 1983) may be mediated by an increase of 5-HT transmission in the nucleus accumbens. Previous studies with intracerebral injections of fenfluramine or norfenfluramine indicated that the lateral hypothalmus, the neostriatum and the nucleus interstitialis striae terminalis are involved in the depression of feeding caused by fenfluramine in deprived rats (Blundell & Leshem 1973; Broekkamp et al 1975). Assuming that 5-HT mediates the effect of intracerebrally injected fenfluramine, activation of 5-HT mechanisms in different brain areas may be involved in drug-induced inhibition of feeding in different conditions.

In conclusion, the present study provides evidence that the nucleus accumbens may be an important area for the inhibitory effect of 5-HT and '5-hydroxytryptaminergic' drugs on particular types of overeating not associated with nutritional deficits.

This work was supported by the CNR (National Research Council, Rome, Italy), Contract No. 84.01981.04.

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J. Pharm. Pharmacol. 1986, 38: 543–546
Communicated October 21, 1985

Effects of chloroquine and didesethylchloroquine on rabbit myocardium and mitochondria

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The effects of chloroquine and didesethylchloroquine on the rabbit isolated perfused heart and on calcium binding and accumulating ability of the heart mitochondria were investigated. The drugs produced negative chronotropy, negative inotropy and a decrease in coronary flow rate in the isolated perfused myocardium. Both chloroquine and didesethylchloroquine significantly decreased mitochondrial calcium binding and accumulation. These results suggest that the cardiodepressant actions of chloroquine could be due in part to alterations in the calcium accumulating abilities of the mitochondrial membranes, and that didesethylchloroquine, among other metabolites, does contribute significantly to the total observed effect of chloroquine on the cardiovascular system.

The therapeutic use of chloroquine has resulted in death from poisoning by the drug (Nelson & Conlin 1950; Sanghvi & Mathur 1965). This has been attributed to an effect on the cardiovascular system (Sanghvi & Mathur 1965; Michael & Aiwazzadeh 1970); chloroquine produces negative chronotropic and inotropic effects in animals (Ojewole 1976; Sofola 1980). However, there have been no reports on the molecular basis of action of chloroquine on either the myocardium or any part of the cardiovascular system. Also, there have been no studies on the contribution of some or all of its metabolites to the action. Since subcellular membranes have been considered to play a role in the regulation of heart function and since alterations in their activities are believed to be involved in the development of myocardial contractile failure (Dhalla et al 1977), the present experiments were undertaken to examine the actions of chloroquine on the isolated perfused myocardium and the subcellular organelles invested with double membranes.

In working with the mitochondria, we took advantage of the configurational changes and the attendant changes in light scattering which result from the binding of Ca^{2+} ions to the mitochondrial inner membrane (Kirtland & Baum 1972).

Methods

In one set of experiments, rabbits, 1·0–1·5 kg, were

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decapitated and the hearts quickly removed and maintained in ice-cold perfusion medium and set up for coronary perfusion by The Langendorff technique. Drugs were administered as bolus injections. The perfusion medium was a modified Tyrode solution containing NaCl 0·9, KCl 0·042, $CaCl_2$ 0·024, $NaHCO_3$ 0·05 and glucose 0·20%. The pH was maintained at 7·4 with bicarbonate. The medium was equilibrated with a gas mixture of 95% O_2 and 5% CO_2 and maintained at 32 ± 1 °C. The myocardium was maintained under a resting tension of 2 g. The contractile force and the heart rate were recorded on the Ugo Basile microdynamometer. The control heart rate was 80 ± 10 beats min^{-1}. The rate of coronary flow was also monitored. The control coronary flow rate was 10± 3 ml min^{-1}.

In another set of experiments, rabbit hearts, chilled in ice-cold homogenizing solution, were trimmed of fat and connective tissue and the ventricles cut into small pieces and homogenized with 5 vol of freshly prepared unbuffered sucrose solution (pH 7·4) containing 1 mM EDTA (ethylenediaminetetraacetate). The homogenate was centrifuged at 800*g* for 20 min to remove cell debris. The supernatant was then centrifuged at 8000*g* for 30 min to obtain the mitochondrial pellet. This was washed twice with the sucrose medium without EDTA, and used within 7 min of isolation. This procedure for isolating mitochondria is similar to that described by Sordahl & Schwartz (1967) who showed that mitochondria so prepared undergo no detectable loss in the calcium binding and uptake activity for at least 3·5 h.

The protein concentration was determined by the Buiret method. Between 160 to 200 μg ml^{-1} of protein (mitochondria) was used for each experiment. Cytochrome C oxidase (the marker enzyme for mitochondria) was determined according to Smith & Camerino (1963) to ascertain the degree of purity of the mitochondria. The activity of cytochrome C oxidase was found to be 1086 ± 68 nmol cytochrome oxidase (mg protein)$^{-1}$ min^{-1}.

To study the effect of binding and uptake of calcium on the mitochondria, the changes in their light scattering properties caused by the addition of 0·16 mM Ca^{2+} ions were determined by ultraviolet-visible absorbance decrease, measured at 520 nm. The mitochondria were suspended in 3 ml of 0·25 M sucrose (pH 7·4; 30± 1 °C) and measurement was as described by Kirtland & Baum (1972).

Chloroquine and didesethylchloroquine (5×10^{-5} M each) were added to the mitochondrial suspension in separate experiments and their effects on calcium binding determined as described above. The test drugs were added 1 min before the addition of calcium. This procedure was repeated for mitochondria (160–200 μg (mg protein)$^{-1}$) suspended in 3 ml of buffered sucrose (0·25 M sucrose containing 5·0 mM pyruvate and 2·5 mM malate, pH 7·4) for the determination of the effects of the test drugs on calcium uptake.

Drug sources

Chloroquine phosphate was supplied by Lab. Aguettant (Germany). Didesethylchloroquine was prepared and characterized in our laboratories (chromatography, ultraviolet spectrophotometry, NMR and mass spectrometry).

Statistics

Student's unpaired *t*-test was used to determine any significant differences between the measured behaviours of the rabbit myocardium and mitochondria under the influence of the test drugs and their behaviours in control medium ($P < 0·05$).

Results and discussion

Myocardial contractility and coronary flow. After application of a dose of the drug, the effect on heart rate and contractility was observed for 20 min. Fig. 1 shows the relationship between the effects of the drugs on heart rate and contractility. The agents inhibited inotropy dose-dependently; 5×10^{-7} M of the drugs had no effect on the heart rate whereas at 5×10^{-5} M there was a slowing of the heart rate (Fig. 1).

Fig. 2 shows the effect of the various concentrations of the drugs on coronary flow. Chloroquine and its metabolite produced a dose-dependent inhibition.

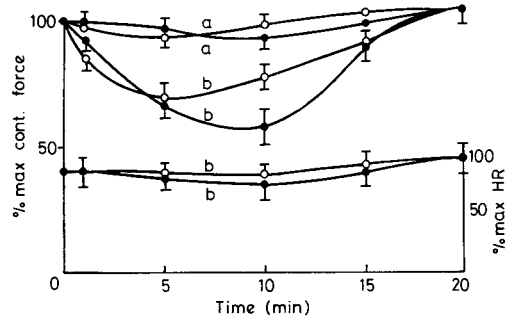

FIG. 1. Changes in ventricular contractility and heart rate induced by chloroquine (○——○) and didesethylchloroquine (●——●) during perfusion of the rabbit isolated heart. Six heart preparations were used for each drug. The vertical bars represent the standard error of the mean. The decrease in contractility produced by these drugs at 5×10^{-5} M dose level were found to be statistically significant ($P < 0·05$).

Effect of drugs on calcium-induced mitochondrial light scattering. In another set of experiments the effects of chloroquine and didesethylchloroquine on the non-energized calcium binding ability of the heart mitochondria were investigated (Fig. 3). Fig. 4 shows the effect of the drugs on energized calcium accumulation by the mitochondria suspended in 0·25 M sucrose (pH 7·4) containing 5·0 mM pyruvate and 2·5 mM malate. In both buffered and unbuffered sucrose media the addition of 0·16 mM calcium ions caused a small decrease in light scattering, as measured by absorbance changes at

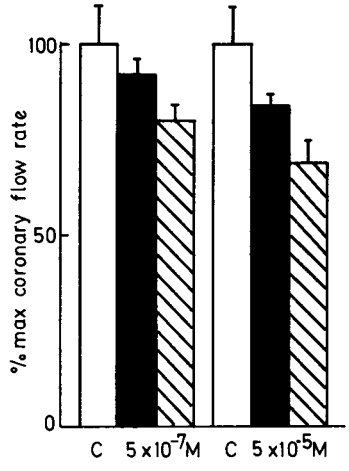

FIG. 2. Effect of chloroquine and didesethylchloroquine on coronary flow rate during perfusion of rabbit isolated heart. Six heart preparations were used for each drug. The vertical bars represent the standard error of the mean. The decrease in coronary flow rate produced by 5×10^{-5} M didesethylchloroquine was found to be statistically significant ($P < 0.05$).

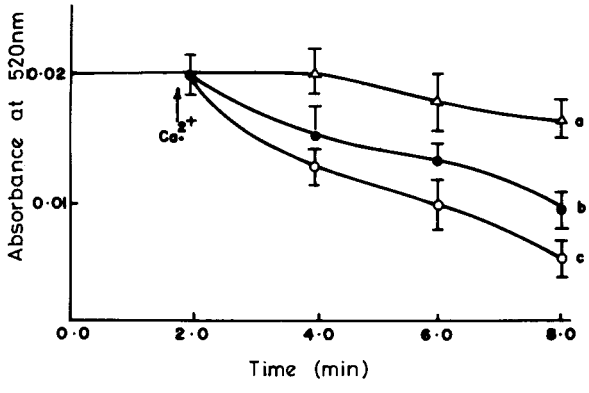

FIG. 3. Effect of 5×10^{-5} M chloroquine (●——●), and didesethylchloroquine (○——○), in non-energized conditions, on $CaCl_2$-induced changes in light absorbance of mitochondrial suspension at pH 7·4. Arrow indicates point of addition of calcium. (a) Control, (b) preincubation with chloroquine (5×10^{-5} M) for 1 min before addition of calcium; (c) preincubation with didesethylchloroquine (5×10^{-5} M) for 1 min before addition of calcium. Six preparations were used for each determination. The absorbance decreases produced by the drugs were found to be statistically significant ($P < 0.05$).

520 nm. The net fall in absorbance following the addition of calcium was markedly potentiated in the presence of chloroquine and didesethylchloroquine.

The results indicate that chloroquine and didesethylchloroquine are potent myocardial depressants; didesethylchloroquine appears to be the more potent. The results from isolated perfused myocardium correlated well with the results of the light scattering studies on mitochondria. The absorbance decreases

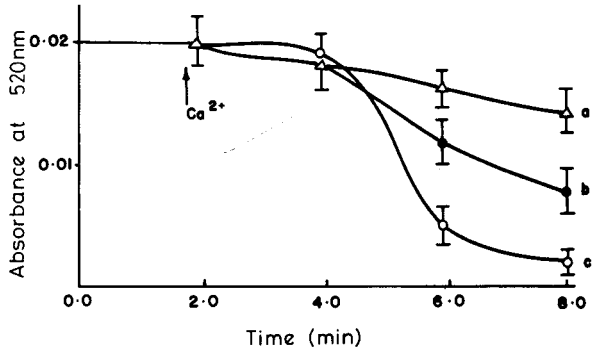

FIG. 4. Effect of chloroquine and didesethylchloroquine on light absorbance changes induced by calcium in mitochondria under energized conditions. Arrow indicates the addition of calcium. (a) Control; (b) preincubation with chloroquine (5×10^{-5} M) for 1 min before addition of calcium; (c) preincubation with didesethylchloroquine (5×10^{-5} M) 1 min before addition of calcium. Six preparations were used for each determination. The absorbance decreases produced by the drugs were found to be statistically significant ($P < 0.05$).

produced by the drugs in the calcium binding and uptake (energy-dependent calcium accumulation) studies were statistically significant ($P < 0.05$). The reduction in coronary flow was a probable consequence of cardiodepression.

Calcium is considered to be the final mediator in the excitation-contraction coupling process (Ebashi & Endo 1968; Langer 1968) and mitochondria, the microsomes and sarcolemma are believed to participate in calcium regulation (Dhalla et al 1977). In addition to playing a role in the generation of ATP through the process of oxidative phosphorylation, the mitochondria have also been reported to accumulate calcium by an energy-independent mechanism (Schuster & Olson 1974; Sordahl 1975).

The decrease in light absorbance of the mitochondria in the presence of chloroquine and didesethylchloroquine was probably due to inhibition of mitochondrial calcium binding or accumulation by these drugs. In their presence, the mitochondrial membrane is expanded, swollen and disrupted, with a consequent increase in mitochondrial transparency and a marked decrease in light absorbance. Under these conditions there is loss of Ca^{2+} and subsequent biomembrane expansion by chloroquine (Ette et al 1981).

Biochemical mechanisms of Ca^{2+} movements and chloroquine effect. Chloroquine is an amphiphilic amine which is cationic at physiological pH. It has been shown by NMR spectroscopy to interact strongly with certain polar lipids resulting in complexes formed by hydrophobic and electrostatic forces and ion-pair formation. Phospholipids in these complexed and relatively electrically neutral forms are protected against enzymatic attack of phospholipases which require the substrate to

be present in a negatively charged form (Seydel & Wasserman 1976). The removal by charge neutralization with chloroquine of sialic acid residues would reduce the binding of calcium to the biomembranes and thus myocardial contractility (Langer 1968). Chloroquine is also a potent inhibitor of phospholipase A and C (Matsuzawa & Hostetler 1980) and its action on phospholipase C would prevent the breakdown of phosphotylinositol PI to phospholidate PA (Michell 1975), which would result in an inhibition of calcium binding and uptake. The desethylated metabolite would behave similarly.

Pathological considerations

The inhibition of mitochondrial calcium binding and uptake by the drugs observed as large-amplitude decreases in light absorbance by the mitochondria, occur because of the link between Ca^{2+} binding/uptake with oxidative phosphorylation and the conformational state of the mitochondria. The absorbance decrease can be related to the orthodox conformation by the mitochondria. Such large-amplitude decreases in light absorbance have been associated with mitochondrial swelling and pathological states such as heart failure (Sulakhe & Dhalla 1971). A probable inhibition of calcium uptake and binding by the drugs would inhibit mitochondrial metabolism in drug-treated myocardia, thereby impairing availability and utilization of energy. This agrees with the observation that chloroquine inhibits mitochondrial oxidation (Stell & Thomas 1972), and damages myocardial mitochondria, causing them to assume the orthodox conformation (Ayiteh-Smith & Gbewonyo 1977).

So the myocardial depression produced by chloroquine and didesethylchloroquine may reasonably be assumed to be due to their inhibitory effect on calcium metabolism (prevention of phosphatidylinositol hydrolysis) in the biomembranes involved in excitation-contraction coupling. The consequence of this inhibitory effect is calcium deficiency. Both calcium deficiency and excess elicit massive disruption in cellular membrane integrity (Crevey et al 1978). Chloroquine and its desethylated metabolites have been shown to produce excessive disruption of mitochondria and to a lesser extent the myofibrils in skeletal muscle exposed to half the normal amount of calcium in-vitro. The damage was reversed by increased calcium levels (25% above the normal concentration; Ette & Essien 1986).

With myocardia treated with chloroquine and didesethylchloroquine, it is suggested that the myocardial depression is due to the inhibitory effects of these drugs on either calcium influx (calcium binding and uptake by the biomembranes involved in excitation-contraction coupling) or release, or their inhibitory effects on both processes.

The greater potency of didesethylchloroquine in producing cardiodepression, decrease in mitochondrial calcium binding and accumulation, could be due to its molecular structure which because of its orientation in the biophase, allows the two hydrogen atoms of the NH_2 group to be involved in additional hydrogen bonding with the biomembrane thereby increasing adsorption above that of the parent drug with a consequent higher degree of displacement of calcium from binding sites. It is therefore pertinent to chloroquine medication that the probable contributions of didesethylchloroquine and other metabolites to the actions of chloroquine should be considered before long term medication is undertaken, as for rheumatoid arthritis. Whilst caution needs to be exercised in extrapolating the results of this study to man, complete heart block in man and dog due to acute chloroquine poisoning has been reported by Michael & Aiwazzadeh (1970).

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