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An in-vitro study of chloroquine transport in the rat submaxillary gland

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Abstract—The uptake and efflux of chloroquine by the rat submaxillary gland in-vitro were studied under various incubation conditions. Variations in the extracellular pH significantly affected both the uptake and efflux of the drug. Increasing chloroquine concentration significantly decreased the uptake. Uptake was also decreased significantly ($P < 0.05$) when compared with control conditions (pH 7·40, 37·5°C, O_2 aeration, 6×10^{-6} M chloroquine) by the following experimental variations: aeration of the incubation medium with N_2 instead of O_2; decrease of bath temperature from 37·5 to 4°C; addition of metabolic inhibitors, cyanide (10^{-3} M), iodoacetic acid (10^{-3} M) and *o*-nitrophenol (10^{-3} M). Cimetidine (10^{-3} M), a known organic cationic inhibitor, had no significant effect on chloroquine uptake when compared with control values. These results show that the uptake of chloroquine by the rat submaxillary gland in-vitro is concentration-dependent and this is indicative of possible saturable binding sites for the drug in the gland. These results suggest that the transfer of chloroquine across the submaxillary gland may be mediated by an active transport process. On the other hand, it is possible that the apparent active transport process implicated in this study could be a consequence of chloroquine ion trapping in the acidic interior of lysosomes.

Both in-vivo (Schaffer et al 1958; Kuroda 1962) and in-vitro (Allison et al 1965; Sams & Epstein 1965) studies have shown that chloroquine accumulates in different organs and tissues of the body. The drug has also been shown to be excreted in human saliva (Ogunbona et al 1986a). A further study on the salivary excretion of chloroquine in man has shown that the saliva: plasma concentration ratios of chloroquine were much higher than unity and these values could not be explained by variations in the saliva pH (Ogunbona & Onyeji, unpublished data). The high tissue:plasma ratio of chloroquine does not necessarily indicate that the drug is trapped inside cells by an active process but that it could also be due to a passive process involving binding of the drug to tissue constituents. This assertion has been corroborated by the results of the study of in-vitro binding of chloroquine to rat muscle preparation (MacIntyre & Cutler 1986). It was found that in spite of the high binding of chloroquine to muscle tissues, the structural integrity of the cell is not essential (i.e. absence of an active process) for accumulation of chloroquine in the muscle.

However, another study by the same authors (MacIntyre & Cutler 1988) demonstrated that the mechanism for the accumulation of chloroquine by rat isolated hepatocytes was predominantly dependent on the structural integrity of the cell. It was concluded that lysosomal ion trapping was responsible for the accumulation.

In the present study, the interaction of chloroquine with rat submaxillary gland in-vitro was investigated with a view to elucidating the mechanism of movement of the drug across the gland. If the transport is not passive, other drugs and compounds, acting as inhibitors, could affect the transfer of chloroquine into the saliva. In that case, the utility of saliva levels of chloroquine in therapeutic drug monitoring (Ogunbona et al 1986a), would need cautious interpretation.

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Materials and methods

Incubations. Submaxillary glands of male Wistar rats, 200–250 g, were removed by blunt dissection from the anaesthetized animals (urethane, 600 mg kg^{-1}, i.p.). The glands were cut into slices weighing 40–80 mg and approximately 0·4 mm thick. Krebs-Ringers Tris (KRT) buffer was the incubation medium, continually aerated and maintained at a temperature of 37·5°C unless stated otherwise. The incubation procedure is essentially the same as that used by Putney & Borzelleca (1971).

Uptake experiments. The gland slices were allowed to equilibrate with the KRT buffer (5 mL) for 1 h. Chloroquine solution was added to obtain a KRT-chloroquine solution of 6×10^{-6} M. After the 1 h equilibration period, incubation was carried out for 0·25, 0·5, 1·5, 2 and 3 h. To measure the uptake of chloroquine, the slices were removed, dipped in four successive washes of KRT, blotted and weighed. The slices were then transferred to test tubes containing 2 mL 0·2 M HCl and shaken vigorously for 1 h. The concentrations of chloroquine in the acidic extracts and the incubation media were determined by a sensitive HPLC technique (Ogunbona et al 1986b). Uptake was calculated as

$$\text{uptake (mL g}^{-1}) = \frac{\text{chloroquine in tissue (ng)}}{\text{wt of tissue (g)/chloroquine concn in incubation medium (ng mL}^{-1})} \quad (1)$$

All other uptake experiments were designed with an initial 1 h equilibration period and 1 h incubation period.

The uptake of chloroquine of different concentrations ranging from 0·6 to 6000×10^{-6} M was investigated. Also the uptake of the drug (6×10^{-6} M) was determined at pH 6·4, 7·4 and 8·4. The uptake at pH 7·4 was also measured under various conditions such as decreasing the bath temperature from 37·5 to 4°C, aeration of the incubation medium with nitrogen instead of oxygen and addition of metabolic inhibitors into the incubation medium. These inhibitors were cyanide (10^{-3} M), *o*-nitrophenol (10^{-3} M) and iodoacetic acid (10^{-3} M). The uptake was also determined in the presence of cimetidine (10^{-3} M) which is a known organic cationic transport inhibitor.

Efflux experiments. After 1 h equilibration, the submaxillary gland slices were incubated with 6×10^{-6} M chloroquine for 1 h. Each tissue slice was then transferred to a series of 12 vials containing 2 mL of KRT buffer for 2 min.

At the end of the series of washouts each tissue was extracted with 2 mL 0·2 M HCl. The concentrations of chloroquine in the extract and in each vial were determined by the HPLC method. The data were computed as the percentage of chloroquine remaining in the slice, and a semi-logarithmic plot obtained (desaturation curves (Shanes & Bianchi 1959)). The rate constant of the efflux was obtained by linear regression analysis of the 10- to 24-min period. The efflux experiments were also performed at a washout pH of 6·4 and 8·4.

Results

Fig. 1 shows results of the uptake of chloroquine (6×10^{-6} M) after different periods of incubation. Each point represents the

mean of ten determinations ± s.e.m. The uptake of chloroquine by the rat submaxillary gland at different chloroquine concentrations is shown in Fig. 2. Each point represents the mean ± s.e.m. of twelve determinations.

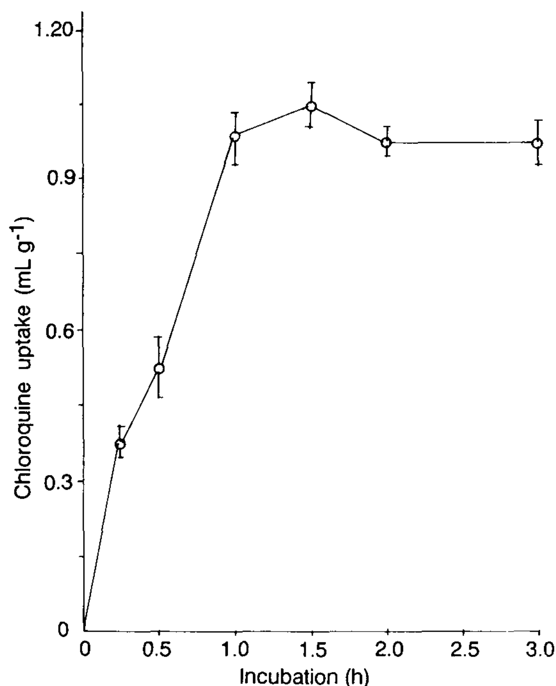

FIG. 1. Mean uptake of chloroquine by rat submaxillary gland slices after varying periods of incubation. Error bars, ± s.e.m.

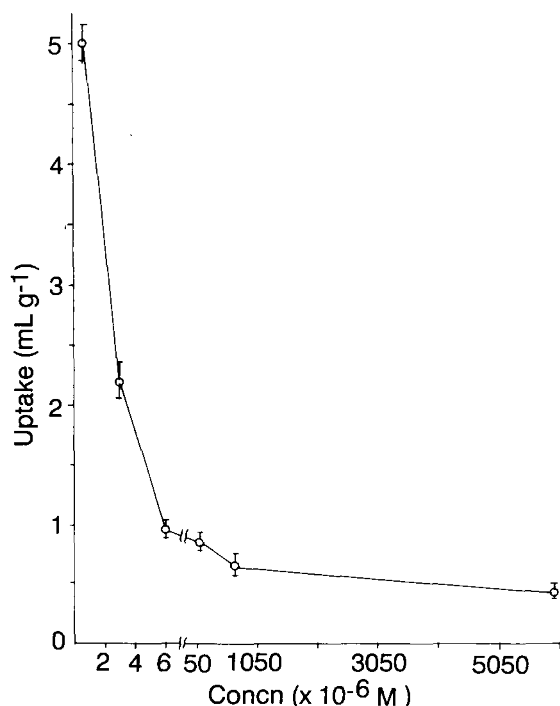

FIG. 2. Mean uptake of chloroquine by rat submaxillary gland slices following incubation with varying concentrations of the drug. Error bars, ± s.e.m.

The uptake of the drug determined at pH 6·4, 7·4 and 8·4 were 0·64 ± 0·04 (n = 12), 0·95 ± 0·14 (n = 24) and 1·34 ± 0·08 (n = 12) mL g^{-1} ± s.e.m., respectively. These values are significantly different ($P < 0·05$) from each other.

The data obtained from incubation of chloroquine under different experimental conditions are summarized in Table 1.

The results of the efflux experiments are shown in the typical desaturation curves presented in Fig. 3. The rate constant of the efflux when the washout was performed in a pH 7·4 KRT buffer was 0·031 ± 0·004 (n = 4). When the washout pH was decreased to 6·4 the rate constant of the efflux significantly increased to 0·069 ± 0·007 min^{-1} ($P < 0·05$). On the other hand, increasing the washout pH from 7·4 to 8·4 resulted in a significant decrease (0·018 ± 0·002 min^{-1}) in the efflux rate ($P < 0·05$).

Table 1. Effect of various conditions of incubation on the uptake of chloroquine by rat submaxillary gland slices.

Incubation condition	n	Uptake (mL g^{-1} ± s.e.m.)	*P*
Control*	24	0·95 ± 0·14	
4°C	12	0·56 ± 0·06	<0·05
N_2	13	0·49 ± 0·06	<0·05
o-Nitrophenol (10^{-3} M)	12	0·43 ± 0·05	<0·05
Iodoacetic acid (10^{-3} M)	13	0·53 ± 0·03	<0·05
Cyanide (10^{-3} M)	12	0·48 ± 0·04	<0·05
Cimetidine (10^{-3} M)	14	0·88 ± 0·05	>0·05

* Control conditions: 6×10^{-6} M chloroquine, pH = 7·40, 37·5°C bath temperature and oxygen aeration.

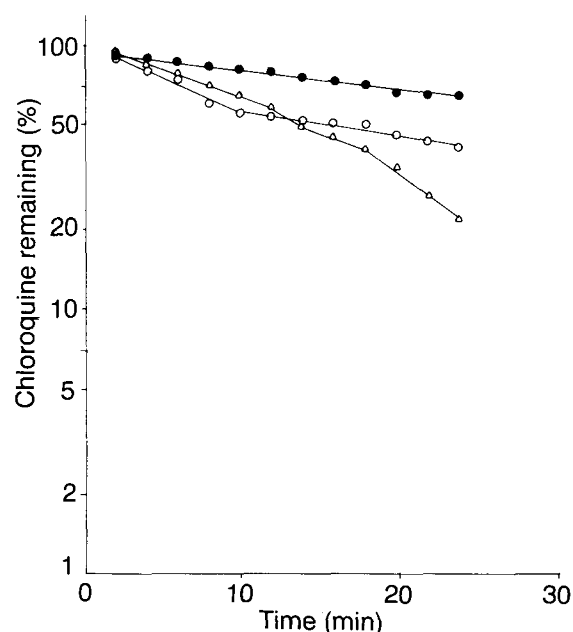

FIG. 3. Typical desaturation curves showing the efflux of chloroquine from submaxillary gland slices of rat following incubation with the drug. Efflux at different pH values, 7·4 (○), 6·4 (△) and 8·4 (●).

Discussion

The experiments were designed with an initial 1 h equilibration period of the submaxillary gland slices with the KRT buffer, because previous studies have shown that cation flux and total tissue water became stable within this time (Putney & Borzelleca 1971). The data in Fig. 1 on the uptake of chloroquine in-vitro

showed that a steady state was reached after one hour incubation of the submaxillary gland slices with the drug. This result is comparable with the finding on the in-vitro uptake of salicylic acid by rat submaxillary gland (Putney & Borzelleca 1971). However, a study of the mechanism of transport of diphenylhydantoin in the rat submaxillary gland in-vitro showed that a steady state was not reached in the uptake of the drug (Allen et al 1975). Since the uptake of chloroquine remained stable after 1 h incubation, all other uptake and efflux experiments were thus designed with an initial 1 h equilibration period and 1 h incubation period.

Fig. 2 shows that the uptake of chloroquine by the gland decreases with increase in the drug concentration. This may be an implication of existence of saturable binding sites for uptake or active uptake of the drug. This result contrasts with the observation that in the in-vitro binding of chloroquine to rat muscle preparation the uptake of the drug was not concentration-dependent (MacIntyre & Cutler 1986). It was suggested by those workers that active transport was not involved.

Alternatively, it is possible that the concentration dependence of chloroquine uptake observed in this study could be due to the lysosomotropic properties of chloroquine (Allison et al 1965) which, as a basic drug, causes a rapid increase in intralysosomal pH, thereby reducing its own accumulation. This is consistent with the conclusion of MacIntyre & Cutler (1988) on the role of lysosomes in hepatic accumulation of chloroquine. All other uptake experiments in this study were performed with a chloroquine concentration of 6×10^{-6} M, well below the toxic plasma chloroquine level of 50×10^{-6} M (Robinson et al 1970). Variation in the pH of the incubation medium significantly affected the uptake of chloroquine. This suggests that the degree of ionization of chloroquine plays a role in the extent of uptake of the drug by the salivary gland. This might not be unexpected since chloroquine is a dibasic compound with pKa values of 8·1 and 10·1 (Irvin & Irvin 1947), and it has been noted that the degree of ionization of basic drugs with pK_a values over 5·5 is profoundly affected by minor alterations of pH and this will reflect on the uptake of the drug by salivary gland (Mucklow et al 1978). Cimetidine has been shown to be an organic cationic inhibitor in a similar manner as probenecid which has been established as an inhibitor of organic anion transport (Somogyi 1987). However, cimetidine has only been shown to inhibit transport of organic cations in the renal proximal tubule. In spite of the high degree of ionization of chloroquine at pH 7·4, cimetidine did not affect its uptake. This could indicate that the organic cationic transport in the renal proximal tubule and in the salivary gland might not have similar specificity.

The results of the efflux experiments show that just as the uptake was significantly influenced by pH, the efflux rate was equally affected, showing that salivary pH is one of the most important factors influencing the concentration of chloroquine in human saliva.

The variety of conditions under which the incubations were performed (Table 1) are known to inhibit energy-dependent processes. All these incubation alterations, except the addition of cimetidine, produced a significant inhibition of the uptake of chloroquine, suggestive of evidence of active transport process in the uptake of chloroquine.

We also considered the phenomenon of lysosomal chloroquine ion trapping. Metabolic inhibitors cause an increase in intralysosomal pH, apparently due to inhibition of energy-dependent active transport of H^+ ions which maintains the low pH within the lysosome (Ohkuma & Poole 1978). This, therefore, would lead to a reduction in chloroquine uptake.

The results of these studies suggest the apparent implication of an active process in the transport of chloroquine into the saliva and the possible existence of a saturable binding site for the drug in the salivary gland. It is also possible that this transport mechanism involves the trapping of chloroquine ion by the lysosome of the gland. Therefore, it is possible that some medicinal agents could inhibit chloroquine excretion in saliva thus rendering unreliable any drug monitoring of chloroquine using the saliva levels.

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