COMMUNICATIONS

An analysis of the inhibitory effects and of possible prostaglandins antagonism of chloroquine in the guinea-pig isolated ileum

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Chloroquine is an antimalarial drug that has been used in the treatment of rheumatoid arthritis and lupus erythematosus because of its antirheumatic properties (Lockie, 1972; Thompson & Werbel, 1972; Rollo, 1975). These properties are clinically different from those of classical steroidal and non-steroidal anti-inflammatory drugs mainly as they appear only after several months of treatment. Its mode of action is still not well understood.

It has been suggested that it could act in systemic lupus by binding to nucleic acids, preventing DNA repair, blocking DNA unwinding and inhibiting DNA and RNA polymerases (Lockie, 1972; Rollo, 1975).

It has been shown to inhibit prostaglandin (PG) synthesis, but only in certain experimental conditions (Greaves & MacDonald-Gibson, 1972; Collier, 1974). Recently it has been demonstrated to act as a PG antagonist in a rat mesenteric vascular bed preparation (Manku & Horrobin, 1976a, b).

We have shown that chloroquine inhibits the isometric contractions of the guinea-pig ileum induced by electrical coaxial stimulation according to Paton (1955), in the same manner as do non-steroidal and steroidal anti-inflammatory drugs (Famaey, Fontaine & Reuse, 1975). This inhibition is reversed by small concentrations of PGs, as also happens with inhibition by non-steroidal anti-inflammatory drugs of isotonic contractions of the guinea-pig ileum to exogenously added acetylcholine, histamine, nicotine (Famaey, Fontaine & Reuse, 1977a) and 5-HT (Famaey, Fontaine & Reuse, 1977c). Moreover, we have recently demonstrated that steroidal and non-steroidal anti-inflammatory drugs at high concentrations are able to specifically antagonize guinea-pig ileum contractions to PGE_1 and $PGF_{2\alpha}$ (Famaey, Fontaine & Reuse, 1977b).

We decided to control the possible PG antagonism of chloroquine on the same preparation by comparison with its effects on contractions to acetylcholine, histamine, nicotine and 5-HT.

Contractions to PGE_1 (5 ng ml^{-1}, 45 s contact time, every 6 min), to $PGF_{2\alpha}$ (20 ng ml^{-1}, 45 s contact time, every 6 min), to acetylcholine (20 ng ml^{-1}, 30 s contact time, every 3 min), to histamine (30 ng ml^{-1}, 30 s contact time, every 3 min), to nicotine (0·5 μg ml^{-1}, 45 s contact time every 6 min) and to 5-HT (30 ng ml^{-1}, 45 s contact time, every 9 min) were elicited on guinea-pig ileal segments of 4 cm length (removed at least 10 cm from the caecum) set up in Krebs Henseleit solution at 37° and gassed with a mixture of 5% CO_2 in oxygen.

Chloroquine at 5 μg ml^{-1} (the lowest concentration reducing the electrically induced contraction by at least 50%, Famaey & others, 1975), was added to the bath after 3 reproducible contractions of each agonist and the ileum was challenged again with the same substances at the same intervals. After 12 min contact time (18 min for 5-HT contractions) chloroquine was washed out from the bath and the ileum was again challenged at three consecutive intervals.

In another series of experiments conducted simultaneously in a similar way, small amounts of PGE_1 (2 ng ml^{-1}) were added to the bath 6 min after chloroquine (9 min for 5-HT contractions) in an attempt to reverse the chloroquine inhibition and were washed out from the bath 6 min (or 9 min for 5-HT) later, with chloroquine.

Chloroquine diminished contractions to PGE_1 and to $PGF_{2\alpha}$ respectively by 52·8% (±9·5) and by 32·4% (±11·0) and this effect was reversed after washing out chloroquine from the bath (mean ± s.e.m., n = 6, $P \leqslant 0{\cdot}01$ and $\leqslant 0{\cdot}05$ respectively, Student's t-test for paired data).

This inhibition occurred at a concentration known to inhibit both bacterial and mammalian cell growth (Rollo, 1975), to affect PG synthesis (Greaves & MacDonald-Gibson, 1972; Collier, 1974) and to inhibit the pressor response to noradrenaline and angiotensin in the rat vascular mesenteric bed, but it was higher than those described by Manku & Horrobin (1976a) as antagonizing the PGE_2 effect in the same rat vascular preparation.

Moreover, the same concentration of chloroquine similarly diminished the ileal responses to acetylcholine by 50·6% (±7·9) to histamine by 60·6% (±8·5), to nicotine by 28·4% (±7·3) and to 5-HT by 31·0% (±8·2) as well as to electrical stimulations by 69·6% (±14·0) (mean ± s.e.m., n = 6, $P \leqslant 0{\cdot}01$ for acetylcholine, histamine and electrical stimulations and $\leqslant 0{\cdot}05$ for nicotine and 5-HT, Student's t-test for paired

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data). No significant statistical differences (Student's *t*-test) were found between these various inhibitions and those of PG-induced contractions. All these inhibitions were reversed by washing out chloroquine from the bath and, as previously demonstrated for electrical stimulations (Famaey & others, 1975) small amounts of PGE_1 were able to restore the contractions to control levels.

It appears from our data that (i) non-specific antagonism was observed between PGs and chloroquine in the guinea-pig ileum, at even higher concentrations than those used in the rat mesenteric vascular bed, (ii) chloroquine behaves like an overall spasmolytic agent on guinea-pig ileal smooth muscle, (iii) this chloroquine inhibition of contractions to acetylcholine, histamine, nicotine, 5-HT and electrical stimulations is reversed by small amounts of PGE_1 added to the bath. This could be due to an inhibition by chloroquine of the endogenous ileal synthesis of PGs which would be necessary for eliciting a normal smooth muscle contraction with all these agonists (including PGs themselves) or more probably, as suggested by us (Famaey & others, 1977b) and others (Chong & Downing, 1973; Bennett, Eley & Stockley, 1975; Schulz & Cartwright, 1976) to a non-specific smooth muscle sensitization induced by PGs to any kind of stimulation. The overall inhibition induced by chloroquine could, in this case, be related to its well known membrane stabilizing properties (Weissmann, 1965) which might affect the smooth muscle membrane reactivity.

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Antidotal action of the oxime HS6 at the soman poisoned neuromuscular junction of the rat and guinea-pig

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Treatment with atropine and oxime is ineffective in animals poisoned by the organophosphorus cholinesterase inhibitor soman (*O*-pinacolyl-methylphosphonylfluoride) (Loomis & Salafsky, 1963; Heilbronn & Tolagen, 1965). This is due to the soman-inhibited acetylcholinesterase rapidly 'ageing' to a form which is resistant to oxime reactivation (Fleisher & Harris, 1965). In addition, the small increase in protection obtained against soman poisoning in animals pretreated with the oximes Toxogonin and P_2S (Wolthuis & Cohen, 1967) suggests that reactivation of the inhibited enzyme is difficult even before 'ageing' has occurred.

The oxime HS6 (1-{2-hydroxyiminomethyl-pyri-

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