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Stimulation of polymorphonuclear leucocyte phospholipase A_2 activity by chloroquine and mepacrine

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Mepacrine is well known as an inhibitor of phospholipase A_2. As such it has been used to assess the physiological and pharmacological importance of this enzyme, in particular its role in the biosynthesis of prostaglandins and related compounds (Table 1). Furthermore the ability of chloroquine, a close analogue of mepacrine, to inhibit phospholipase A_2 and thereby block the formation of all products of the arachidonic acid cascade, may contribute to its effectiveness in the treatment of chronic inflammatory disorders.

In this communication we report on the effects of chloroquine, mepacrine and the related compound, primaquine, on the activity of two sources of phospholipase A_2 activity which may contribute to the pathogenesis of inflammation: (1) a crude phospholipase A_2 activity of rabbit neutrophils, predominantly associated with the lysosomal granules (Fransen et al 1974; Traynor & Authi 1981) and (2) phospholipase A_2 activity present in the peritoneal cavity of the rabbit 17 h after i.p. injection of glycogen or thioglycollate (Fransen et al 1978; Authi & Traynor 1979).

A preliminary account of part of this work has been presented (Authi & Traynor 1979).

Sterile peritoneal exudates were collected from female New Zealand White rabbits 17 h after i.p. injection of thioglycollate medium U.S.P. (Traynor & Authi 1981). The invading leucocytes (95% neutrophils) were collected by centrifugation (200 *g*, 4 min, 20 °C), washed in 0·9% NaCl (saline) and resuspended in Hepes buffer, pH 7·4, at $7{\cdot}4 \times 10^6$ cells ml^{-1}, then sonicated (2×2 min, 6 μm). The preparation was centrifuged (3000 *g*, 10 min, 4 °C) to provide a crude phospholipase A_2 (termed neutrophil sonicate phospholipase A_2) (Traynor & Authi 1981).

The exudate fluid obtained after removal of the leucocytes contained phospholipase A_2 activity (termed peritoneal fluid phospholipase A_2) with similar properties to the enzyme described by Fransen et al (1978), and was used without further purification. Phospholipase A_2 activity was determined as the ability to release [1-^{14}C]oleic acid from the membrane phospholipids of *Escherichia coli* (Traynor & Authi 1981). Incubations were performed at the pH optimum for each enzyme, unless stated otherwise, and conditions (see Figs) were adjusted to give between 20 and 30% hydrolysis of phospholipid substrate in controls.

Other rabbit neutrophil preparations used were [assay conditions]: (i) a crude lysosomal preparation obtained as an 8200 *g* pellet [20 nmoles phospholipid, 0·5 mg protein, 15 min incubation] (Fransen et al 1974), (ii) phospholipase A_2 activity released during phagocytosis [20 nmoles, 0·09 mg, 60 min] (Traynor & Authi 1981) and (iii) a partially purified phospholipase A_2 (supernatant II) obtained by acid-extraction of lysosomal membranes [see Table 2] (Kaplan-Harris & Elsbach 1980).

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Effects on neutrophil sonicate phospholipase A_2 and related preparations

Mepacrine and chloroquine increase the activity of the neutrophil sonicate phospholipase A_2 in a dose-dependent manner (Fig. 1). Mepacrine has approximately ten-fold the activity of chloroquine. Primaquine, however, only produced a slight enhancement of the enzyme activity, but the predominant effect of this compound was inhibitory.

Mepacrine 5×10^{-5} and 10^{-4} M also enhanced the phospholipase A_2 activity of an 8200 *g* neutrophil pellet by 40 and 72% respectively at pH 6·5 and by 14 and 29% at pH 9·0. Similarly, mepacrine (5×10^{-4} M) increased

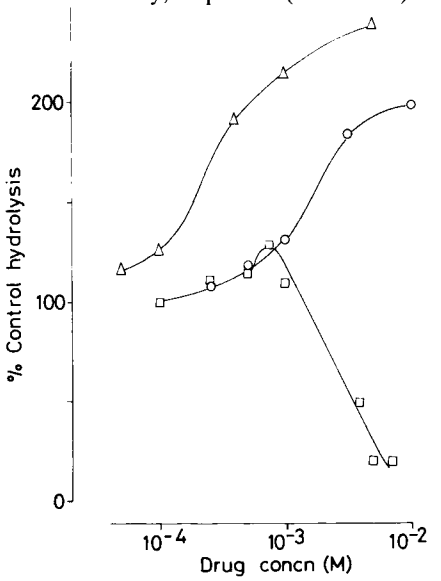

FIG. 1. Effect of chloroquine (○), mepacrine (△) and primaquine (□) on the hydrolysis of [^{14}C]phospholipids of *E. coli* by phospholipase A_2 of neutrophil sonicate. 20nmoles phospholipid, 5 mM $CaCl_2$, 40 mM Tris pH 6·5, 0·1 mg enzyme protein. 30 min 37 °C. Ordinate: % control hydrolysis. Abscissa: drug concentration (M). Each point is the mean of two experiments.

Table 1. Some systems in which inhibition of phospholipase A_2 by mepacrine (or *chloroquine) has been demonstrated.

Tissue	Drug concn (M)	Buffer/ medium	Ref.
Guinea-pig lung	4×10^{-5}	Krebs solution	Vargaftig & DaO Hai 1972
Guinea-pig lung	4×10^{-5}	Krebs solution	Blackwell et al 1978
Guinea-pig lung homogenate	2×10^{-4}	Krebs solution	
Guinea-pig spleen slices	10^{-3}	Krebs solution	Flower & Blackwell 1976
Horse platelets	10^{-4}–10^{-3}	Tris buffer pH 7·4	Lapetina et al 1981
Toad bladder	10^{-6}	Ringer solution	Yorio & Bentley 1978
Intact rabbit neutrophils	10^{-6}–10^{-3}	Gey's solution with Hepes pH 7·4	Hirata et al 1979
* Rat liver lysosomes	10^{-2}†	Sodium acetate buffer pH 4·4	Matsuzawa & Hostetler 1980
* Rat liver lysosomes	10^{-5}–10^{-2}	Sodium acetate buffer pH 5·4	

† Slight stimulation observed at $1{\cdot}25 \times 10^{-4}$ M.

the activity of phospholipase A_2 released by neutrophils during phagocytosis (by 62% at pH 6·0) whilst chloroquine (10^{-3} M) increased this enzyme activity by 47% at pH 6·0, by 66% at pH 7·5 but inhibited activity (−27%) at pH 9·0.

In contrast the drugs inhibited the activity of a partially purified phospholipase A_2 from lysosomal membranes (Table 2).

Effects on peritoneal fluid phospholipase A_2

Mepacrine > primaquine > chloroquine inhibited this enzyme (Fig. 2). With chloroquine the degree of inhibition was seen to vary with the pH of the incubation medium (27% inhibition at pH 6·0, 36% at pH 7·5 and 53% at pH 8.5). These studies used a lower level of substrate than the work with the neutrophil sonicate enzyme. However, when tested at six substrate concentrations over the range 10–70 nmoles the inhibition caused by 4×10^{-4} M mepacrine was constant at 69 ± 2%.

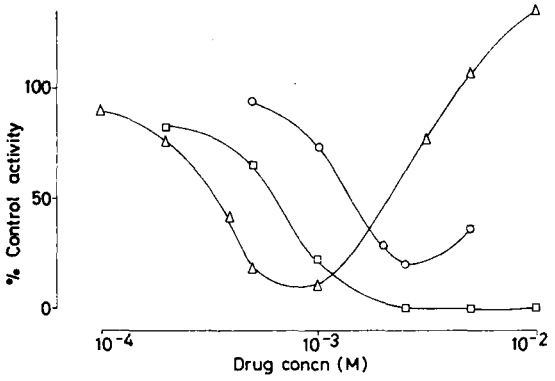

FIG. 2. Effect of chloroquine (○), mepacrine (△) and primaquine (□) on the hydrolysis of [^{14}C]phospholipids of *E. coli* by peritoneal fluid phospholipase A_2. 10nmoles phospholipid, 5 mM $CaCl_2$, 40 mM Tris pH 6·0, 0·5 mg enzyme protein. 5 min 37 °C. Ordinate: % control activity. Abscissa: drug concentration (M). Points represent means of two determinations.

Table 2. Effect of chloroquine-like drugs on the activity of a partially purified phospholipase A_2 derived from neutrophils.

Drug concn	% control response in presence of Chloroquine	Mepacrine	Primaquine
10^{-3}M	97	92	76
5×10^{-3}M	N.T.	42	3
10^{-2}M	71	N.T.	N.T.

10 nmol *E. coli* phospholipid, 5 mM $CaCl_2$, 40 mM Tris pH 6·0, 0·05 mg enzyme protein, 15 min 37 °C. Each value is the mean of two experiments.
N.T. = not tested.

At concentrations of mepacrine higher than those causing maximal inhibition a reversal of effect was seen, resulting in stimulation of activity at 10^{-2} M. The results for chloroquine, but not primaquine, show a similar trend (Fig. 2). In early work with the peritoneal fluid enzyme a stimulation was also observed at low levels of drugs (Authi & Traynor 1979), however these findings could not be repeated on twelve subsequent preparations.

The results we have obtained with chloroquine and mepacrine are contradictory to those reported by other workers though using different systems (Table 1). Such differences could be due to the drug concentrations used, the pH at which the study is performed, the assay method, or the particular phospholipase A_2 involved. The peritoneal fluid enzyme is inhibited although this inhibition is reversed at high doses. However, such doses are higher than generally used. More interesting is the stimulatory response obtained with the neutrophil phospholipase A_2 preparations. This effect occurs at concentrations of drugs, and is retained at pH values used by others (Table 1), and is seen under conditions in which the peritoneal fluid enzyme and the partially purified enzyme derived from lysosomal membranes are inhibited. Moreover, since mepacrine also inhibits the membrane phospholipase A_2 of intact neutrophils and chloroquine inhibits the soluble lysosomal phospholipases A_2 of liver cells (Table 1) the stimulatory response may be confined to soluble lysosomal phospholipase A_2 preparations derived from neutrophils.

In our assays primaquine behaves differently from chloroquine and mepacrine. This is perhaps not surprising since although the compounds do have structural similarities pharmacological differences between primaquine and chloroquine have been noted (Bowman & Rand 1980; Li & Magnus 1972; Kelman et al 1981).

The lysosomal enzymes of neutrophils may have a role in providing prostaglandins in some inflammatory exudates (Anderson et al 1971). The stimulation we have observed is, therefore, surprising since it suggests an ability to promote the production of prostaglandins and related compounds in such exudates, especially since chloroquine has no effect on prostaglandin biosynthesis from arachidonic acid (Greaves & McDonald-Gibson 1972; Stone et al 1975). On the other hand the results may in part explain the reported ability of

chloroquine to potentiate oedema in adjuvant arthritis (Stone et al 1975).

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Inhibition by enkephalins of peristaltic activity of the rabbit ileum and its reversal by naloxone

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Two pentapeptides, the enkephalins have been isolated and identified from the mammalian gastrointestinal tract and have been shown to be potent opiate agonists (Hughes 1975; Lord et al 1977; Hughes et al 1977). Further work has demonstrated that methionine (met-)-enkephalin and D-Ala^2-Leu-enkephalinamide affected the peristaltic reflex of the guinea-pig isolated ileum (van Nueten et al 1977; Kromer et al 1980). On the other hand, the rabbit isolated ileum has been scarcely used as model to study the peristaltic reflex (Feldberg & Lin 1949; Beleslin et al 1978). However, after evidence of the existence of met- and leucine (leu)-enkephalin as well as of enkephalin receptors in the rabbit intestine was reported (Hughes et al 1977; Oka 1980) it became interesting to investigate the action of enkephalins on the peristaltic activity of rabbit isolated ileum.

Method

In these experiments 16 rabbits of either sex, 2–2·5 kg were used. The peristaltic reflex was studied in the isolated ileum, by means of a modified Trendelenburg method (Beleslin et al 1978). Briefly, ileal segments were suspended in Tyrode solution at 37 °C and at pH 7·3 gassed with 95% O_2 and 5% CO_2 in 20 ml organ bath. The intraluminal pressure changes were recorded by means of a float recorder (Stephenson 1948), the volume of fluid expelled was recorded by means of a drop recording unit (silver drop tube, electronic drop recording unit, Thorp impulse counter and time clock) and measured by a measuring cylinder, while the movements of the longitudinal muscle were recorded via an isotonic frontal lever. After both tone and spontaneous rhythmic contractions became stable, the peristaltic reflex was elicited by increasing the intraluminal pressure by 1·5 to 2·5 cm H_2O maintained for the duration of experiment. The organ bath fluid was renewed every 10 min. All substances were added to the bath and therefore acted from the serosal surface of the ileum. The concentrations of enkephalin refer to the peptides, while those of naloxone to naloxone hydrochloride.

When the rabbit isolated ileum is kept under a constant intraluminal pressure of a few cm of H_2O for several hours the peristaltic waves are often frequent during the first 10 to 20 min of an experiment, becoming gradually slower and more regular in most preparations throughout the experiment. When this activity appears, the ileal segment is suitable for studying the effect of drugs that depress the propulsive movements of the intestine.

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