Effects of antimalarial drugs on phospholipase A_2

K.S. AUTHI & J.R. TRAYNOR

Biochemical Pharmacology Laboratory, Department of Chemistry, University of Technology, Loughborough, Leics. LE11 3TU

Antimalarial drugs of the chloroquine-type inhibit lipolytic processes in fat tissue *in vitro* (Markus & Ball, 1969) and phospholipase activity in various tissues (Blackwell, Flower, Nijkamp & Vane, 1978) including the malarial parasite (Cenedella, Jarrell & Saxe, 1969). Several of these drugs have been used in the treatment of rheumatoid conditions. We report the effects of three antimalarial drugs, chloroquine, mepacrine and primaquine, on the activity of a crude phospholipase A_2 enzyme obtained from an inflammatory peritoneal exudate (Fransen, Dobrow, Weiss, Elsbach & Weglicki, 1978).

Phospholipase A_2 activity was assayed against E. coli labelled with [1-^{14}C]-oleate. Greater than 95% of the incorporated label was in the 2-position of membrane phospholipids. Radiolabelled E. coli were autoclaved for 15 min at 2.7 kg/cm^2 to inactivate endogenous bacterial phospholipases and render the membrane more susceptible to enzymic attack. Assays were performed at pH 6.0 in tris buffer 4×10^{-2} M containing calcium 5×10^{-3} M at 37°C for 5 min. Lipid products were extracted, separated by TLC and areas of plates containing radioactive lipids were scraped off and radioactivity determined by scintillation counting.

All three drugs inhibited the enzymic hydrolysis of E. coli phospholipids. IC_{50} for mepacrine was 33×10^{-5} M, primaquine was twice and chloroquine five times less active. In addition the drugs chloroquine and mepacrine showed a stimulation of hydrolysis at approximately 20-fold lower doses, whilst at doses above those causing maximum inhibition a reversal of the inhibitory effects was seen.

The antimalarial drugs examined are amphiphilic cationic drugs. Other drugs of this general type inhibit phospholipase enzymes, possibly by effects on the substrate (Kunze, Nahas, Traynor & Wure, 1976). In relation to this we have studied the effects of the drugs on the stability of guinea-pig red blood cells to hypotonic haemolysis. All three drugs again showed paradoxical dose-dependent inhibitory and stimulatory effects.

The findings described may be of relevance to the use of the drugs in the treatment of malaria and rheumatoid diseases and in their effects in causing drug-induced lipidoses.

We thank the M.R.C. for support.

References

BLACKWELL, G.J., FLOWER, R.J., NIJKAMP, F.P. & VANE, J.R. (1978). Phospholipase A_2 activity of guinea-pig isolated perfused lungs: stimulation and inhibition by anti-inflammatory steroids. *Br. J. Pharmac.*, **62**, 78–79.

CENEDELLA, R.J., JARRELL, J.J. & SAXE, L.M. (1969). *Plasmodium berghei*: production *in vitro* of free fatty acids. *Experimental parasitology*, **24**, 130–136.

FRANSEN, R., DOBROW, R., WEISS, J., ELSBACH, P. & WEGLICKI, W.B. (1978). Isolation and characterisation of a phospholipase A_2 from an inflammatory exudate. *J. Lipid Res.*, **19**, 18–23.

KUNZE, H., NAHAS, N., TRAYNOR, J.R. & WURL, M. (1976). Effects of local anaesthetics on phospholipapses. *Biochim. Biophys. Acta*, **441**, 93–102.

MARKUS, H.B. & BALL, E.G. (1969). Inhibition of lipolytic processes in rat adipose tissue by antimalarial drugs. *Biochim. Biophys. Acta*, **187**, 486–491.

The transport of cimetidine across the rat small intestine *in vitro*

H.E. BARBER, G.M. HAWKSWORTH & S.J. TURNER

Department of Pharmacology and Department of Therapeutics and Clinical Pharmacology, University Medical Buildings, Foresterhill, Aberdeen, AB9 2ZD.

Cimetidine, an H_2-receptor blocking drug, is well absorbed in man, rat and dog after oral dosing (Taylor & Cresswell, 1975; Burland, Duncan, Hesselbo, Mills, Sharpe, Haggie & Wyllie, 1975). However, absorption is often discontinuous and does not follow first-order kinetics. The aim of this study was to investigate whether cimetidine is actively absorbed across the small intestine of the rat.

The everted-sac method, based on that described by Wilson & Wiseman (1954) was used. Sacs were prepared from the small intestine and randomised with respect to position along the intestine. In some experiments the solution on the mucosal side initially contained cimetidine (40 μmol/l, 200 μmol/l, 400 μmol/l) in Krebs-Henseleit bicarbonate-buffered saline (pH 7.2); in other experiments, cimetidine in

Table 1 The transport of cimetidine across rat small intestine *in vitro*

Initial Concentration (μmol/l)	*DNP*	*Serosal Transfer* (nmol/g wet wt/h)	*Serosal/Mucosal Ratio*
40	–	11.9 ± 0.8 (16)**	1.8 ± 0.3 (8)**
40	+	4.1 ± 0.4 (16)**	0.6 ± 0.1 (8)**
200	–	21.2 ± 3.4 (12)*	0.4 ± 0.1 (8)*
200	+	12.0 ± 0.5 (12)*	0.7 ± 0.1 (8)*
400	–	42.9 ± 3.6 (8)	0.6 ± 0.1 (8)
400	+	54.2 ± 7.7 (8)	0.6 ± 0.1 (8)

All values are given as mean ± s.e. mean with the number of determinations in parentheses. * $P < 0.05$, ** $P < 0.001$. Significance values refer to the difference between corresponding results in the presence and absence of DNP using a non-paired *t* test.
Serosal transfer is reported for experiments with cimetidine initially present only on the mucosal side. Serosal/mucosal ratios refer to experiments in which cimetidine was initially present in both solutions.

the same concentrations was present on both the mucosal and serosal sides. 2,4-Dinitrophenol (DNP, 500 μmol/l) was used as an inhibitor of active transport. The sacs were incubated for 1 h at 37°C in an atmosphere of 95% O_2/5% CO_2. Solutions from the mucosal and serosal sides were then extracted and assayed for cimetidine by a high pressure liquid chromatographic method (Randolph, Osborne, Walkenstein & Intoccia, 1977).

At lower concentrations (40 μmol/l and 200 μmol/l), cimetidine transport was inhibited by DNP. DNP also inhibited the transport of [^{14}C]-glucose (400 μmol/l; sp. act. = 248 mCi/mmol) but not of ethanol (4.3 mmol/l), present in the incubations to monitor active and passive transfer respectively. The results in Table 1 indicate an active absorption process for cimetidine observable at lower substrate concentrations which is masked by a diffusion process at higher concentrations (Akedo & Christensen, 1962).

In some experiments cimetidine sulphoxide was detected after incubation and it is possible that this may account for some of the urinary sulphoxide found after oral administration of [^{14}C]-cimetidine (Taylor & Cresswell, 1975).

We should like to thank Dr B. Hawkins and Dr R. Griffiths for their interest in the project and Smith, Kline and French Ltd. for the gift of cimetidine, metiamide and cimetidine sulphoxide.

References

AKEDO, H. & CHRISTENSEN, H.N. (1962). Nature of insulin action on amino acid uptake by the isolated diaphragm. *J. Biol. Chem.*, **237**, 118–122.

BURLAND, W.L., DUNCAN, W.A.M., HESSELBO, T., MILLS, J.G., SHARPE, P.C., HAGGIE, S.J. & WYLLIE, J.H. (1975). Pharmacological evaluation of cimetidine, a new histamine H_2-receptor antagonist, in healthy man. *Br. J. Clin. Pharmac.*, **2**, 481–486.

RANDOLPH, W.C., OSBORNE, V.L., WALKENSTEIN, S.S. & INTOCCIA, A.P. (1977). High pressure liquid chromatographic analysis of cimetidine, a histamine H_2 receptor antagonist, in blood and urine. *J. Pharm. Sci.*, **66**, 1148–1150.

TAYLOR, D.C. & CRESSWELL, P.R. (1975). The metabolism of cimetidine in rat, dog and man. *Biochem. Soc. Trans.* **3**, 884–885.

WILSON, T.H. & WISEMAN, G. (1954). The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. *J. Physiol. Lond.*, **123**, 116–125.

The action of dopamine on constrictor responses in the perfused rat mesenteric artery

N. ANWAR & D.F.J. MASON

Pharmacology Department, St. Bartholomew's Hospital Medical College, London EC1.

Dopamine or apomorphine induced vasodilatation in mesenteric, renal and femoral beds of the anaesthetized dog is mediated by a specific dopamine receptor (Yeh, McNay & Goldberg, 1969; Buylaert, Willems & Bogaert, 1977). Inhibition of stimulation induced noradrenaline overflow by dopamine has been reported for rabbit ear artery (Hope, McCulloch, Rand & Story, 1978), cat spleen and nictitating membrane (Langer, 1973). These and other inhibitory effects of dopamine may be due to an action on an inhibitory presynaptic dopamine receptor (Hope *et*