presence of the drug in the circulation [15] and, therefore, its continuous entry into the tumour. Evaluation of data becomes even more complex when one considers that the kinetics of drug metabolism by the tumour will probably differ according to the drug's rate of entry into the cells which, in turn, could be influenced by the rate of the drug's leakage from liposomes and association with plasma proteins [15].

The therapeutic efficacy of liposomal drugs in tumour-bearing animals has varied [9, 10, 20–26] according to the type of liposomes, the drug and the tumour model used. Our findings suggest that further work with alternative liposomal compositions and sizes which provide for a wider spectrum of rates of drug leakage and vesicle clearance in selected tumour models should improve our understanding of liposomal drug mode of action in experimental cancer chemotherapy and help to establish conditions for its optimization. Unfortunately, SM liposomes, which were so effective under the present conditions, induce, as such, spleen and liver enlargement in chronically treated mice [27]. It would therefore be of interest to see whether cholesterol-rich distearoyl phosphatidylcholine liposomes with a half-life of 20 hr in mice [14] are as effective.

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Division of Clinical Sciences PAMELA LARGE
Clinical Research Centre GREGORY GREGORIADIS*
Watford Road,
Harrow, Middx HA1 3UJ, U.K.

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* To whom all correspondence should be addressed.

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The action of histamine on *p*-nitrophenyl phosphatase activity in cardiac microsomes

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It is generally agreed that the enzyme system ($Na^+ + K^+$)ATPase is involved in the transport of Na^+ and K^+ across cell membranes [1]. The ATPase reaction is a two-step process; the Na^+-dependent formation of a phosphoenzyme, followed by its breakdown in the presence of K^+ [2, 3]. Phosphatase activity associated with the ($Na^+ + K^+$)-ATPase system has been demonstrated using the substrate *p*-nitrophenyl phosphate (pNPP), whose breakdown is stimulated by K^+ and inhibited by ouabain [4, 5]. It seems likely that K^+-activated *p*-nitrophenyl phosphatase (pNPPase) represents the terminal dephosphorylation phase of the ($Na^+ + K^+$)ATPase enzyme system on

the outer surface of the cell membrane [2, 6], and is regarded as a sensitive and specific probe for studies of the myocardial Na^+ pump ATPase system [7]. Its activity may also reflect the contractile state of the heart [8]. Isolated cardiac microsomes accordingly exhibit ATPase-related pNPPase activity [9, 10] and appear closely involved in the regulation of contractile function by controlling intracellular calcium flux. Since histamine increases myocardial contractility with a sensitivity comparable to that of the catecholamines [11], we wished to ascertain whether it might act directly upon cardiac microsomal pNPPase.

Methods

Isolation of cardiac microsomes. Nine normal healthy pigs of either sex and weighing 6–7 kg were anaesthetized as previously described [12], thoracotomy was performed and the beating heart excised. The heart was washed in tap water and cooled in ice. The ventricles and septum were trimmed of fat, diced and homogenized in a Waring blender for 1 min in 3 vols of an ice-cold solution containing 0.32 M sucrose, 0.12 M potassium chloride, 0.1 M *l*-histidine hydrochloride and 5 mM dithiothreitol at pH 7.4. The homogenate was centrifuged at 600 *g* for 15 min, the supernatant strained and centrifuged at 8800 *g* for 20 min, and 30,000 *g* for 15 min, discarding each pellet obtained. The pellet obtained after centrifugation at 100,000 *g* for 1 hr was gently resuspended in fresh homogenizing solution using a hand-operated Teflon homogenizer. The temp throughout the preparative procedure was kept between 0–4°. This microsomal preparation is known to be low in succinic dehydrogenase and β-glucuronidase but possessed some 5'-nucleotidase activity and is known to be capable of oxalate-dependent sequestration of calcium [13], thus indicating enrichment in the sarcoplasmic reticulum and sarcolemma. The protein concn of these suspensions was determined [14] with absorbance measured at 720 nm on a Pye Unicam SP500 spectrophotometer using a tungsten light source with a red filter.

Measurement of pNPPase activity. Cardiac microsomal pNPPase activity was assayed in the presence of 0.12 M potassium chloride, 0.1 mM calcium chloride, 10 mM magnesium chloride, 50 μg microsomal protein, 25 mM pNPP (sodium salt), various drugs as required and 50 mM Tris–HCl buffered to pH 7.1. The total vol was 2 ml and the incubation temp was 30°. The reaction was started by the addition of microsomes, and stopped 30 min later by 2 ml ice-cold NaOH. Under these conditions of incubation the breakdown of pNPP was linear with time. Absorbance was measured at 420 nm and compared against blanks prepared by adding microsomes at the end of the incubation. The pre-incubation of cardiac microsomes with various drugs was carried out at a temp of 30° for 15 min after which aliquots of each mixture were withdrawn to start the pNPPase incubation studies. All reagents were of Analar grade.

Results and discussion

The effect of various concns of histamine hydrochloride on cardiac microsomal pNPPase is shown in Fig. 1. Depression of pNPPase activity was not found to be statistically significant below a histamine concn of 50 μM. Inhibition of activity progressively increased as the concn of histamine was raised. It reached 65.8% at 4 mM. Pre-incubation of histamine with cardiac microsomes did not influence the extent of the depression of pNPPase activity. Lineweaver–Burk analysis of this depression of pNPPase activity (Fig. 2) showed that histamine was acting as a competitive inhibitor. The effect of the histamine H1 antagonist, promethazine hydrochloride, on cardiac microsomal pNPPase is shown in Fig. 3. Statistically significant depression of pNPPase activity was not observed below a concn of 4 mM (−36.9%). However, pre-incubation of cardiac microsomes with promethazine caused this dose–

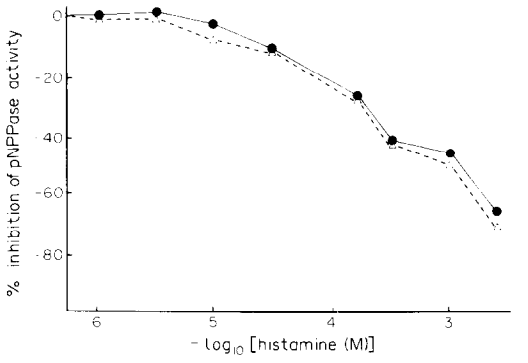

Fig. 1. The effect of histamine on cardiac microsomal pNPPase (circles). Pre-incubation of microsomes with histamine (triangles) produced no difference in response. Concns of histamine of 50 μM and greater produced statistically significant depression ($P < 0.05$; $N = 8$) of pNPPase activity.

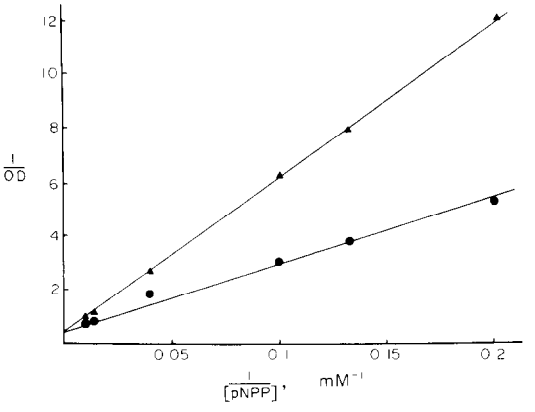

Fig. 2. Lineweaver–Burk plot showing competitive inhibition of cardiac microsomal pNPPase by 1 mM histamine. Enzyme activity is expressed as the reciprocal of O.D. measurements.

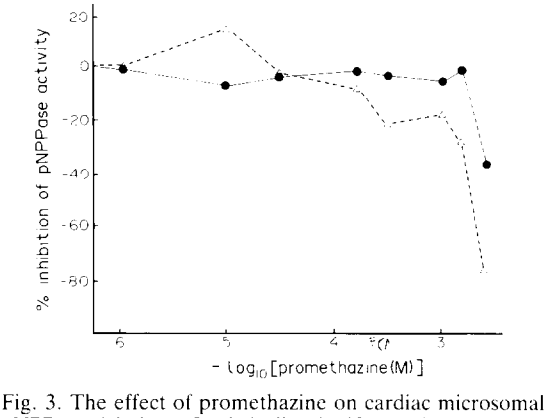

Fig. 3. The effect of promethazine on cardiac microsomal pNPPase (circles). Statistically significant depression of activity ($P < 0.05$; $N = 6$) was observed only at the highest concn tested (4 mM). When promethazine was pre-incubated with microsomes (triangles), the dose–response curve was shifted to the left with significant depression of pNPPase activity being produced by concns of 0.2 mM and above.

response curve to be shifted to the left, with significant depression evident at 0.2 mM. Also, pre-incubation caused inhibition by 4 mM promethazine to be increased from −36.9 to 76.1%.

The effect on cardiac microsomal pNPPase of a number of other agents was also studied. ATP was found to be a competitive inhibitor at millimolar concns, whilst, similarly to promethazine, sodium amytal and thiopentone sodium each inhibited activity only when in the millimolar range. No effect on pNPPase could be demonstrated by any concns of the following agents: imidazole, tyramine, diazepam, ethanol, lactose, glucagon, quinidine, caffeine, theophylline, cyclic AMP, dibutyryl cyclic AMP, and cyclic AMP with theophylline.

The characteristics of this cardiac pNPPase, particularly the competitive inhibition by ATP, are similar to those found in other microsomal preparations [9, 10]. The activity of the enzyme reported here was progressively stimulated by concns of Mg^{2+} above 0.1 mM, whereas it could be partially inhibited by Ca^{2+} above 0.5 mM. The effects of pharmacological intervention on this enzyme have not been studied before.

The present study has reported competitive inhibition of cardiac microsomal pNPPase by histamine. This poses the important question of whether the cardioactivity of histamine [11] may to some extent be attributable to a direct effect on this enzyme system. The pharmacological concns of histamine required for an effect in this study are higher than those to be expected *in vivo* but this is a common problem with *in vitro* experiments and thus no firm conclusions may be drawn. The next logical step is to apply the intact myocyte approach of Liu and Onji [7], since this should determine: (i) whether histamine acts at physiological concns on the sarcolemma, and (ii) whether these effects may be ascribed to the sarcolemma or the sarcoplasmic reticulum, a fact left unclear by the microsomal preparative procedure used in the current study.

The mode of action of histamine on the heart is currently uncertain. Its positive inotropic effects may even be independent of H1, H2 or β-adrenergic receptors altogether [15]. The inotropic actions of histamine are known to be preceded by increases in cyclic AMP [16, 17], but since a number of agents known to stimulate adenyl cyclase had no effect on pNPPase in the current study, one would accordingly be surprised if adenyl cyclase was related to the effect of histamine on microsomal pNPPase. However, since sarcolemmal pNPPase is inhibited by ouabain [4, 5], it is attractive to consider that histamine might act in a similar way, with its positive inotropic action a result of a concomitant change in the Na^+/K^+ balance.

Whether the inhibitory effect on microsomal pNNPase activity of the H1 receptor antagonist, promethazine, was related to H1 or local anaesthetic actions is quite unclear. Two barbiturates at millimolar concns exerted similar effects but quinidine, known for its local anaesthetic properties, did not. The reason why pre-incubation of microsomes with promethazine produced a greater depression of pNPPase activity is also unclear.

In summary, a preparation of pig cardiac microsomes was found to exhibit pNPPase activity. The activity of this enzyme was competitively inhibited both by histamine and ATP. Barbiturates and promethazine, an H1 histaminic antagonist, were also found to inhibit enzyme activity, whereas quinidine did not.

Department of Paediatric Cardiology
Institute of Child Health
London, U.K.

RICHARD K. H. WYSE
MARGUERITE KIRBY

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