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COMMUNICATIONS

The encapsulation of insulin in erythrocytes

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Insulin degrading activity has been demonstrated in kidney, liver, spleen and other tissues including haemolysates of erythrocytes (Tschesche et al 1974). It has been proposed (Ihler et al 1973; Zimmermann 1973) that erythrocytes are possible carriers for use in a sustained release intravenous drug delivery system. In this study we have encapsulated insulin into intact erthyrocytes, but have found some degradation. We have therefore, examined a number of low molecular weight compounds for their suitability as inhibitors of the insulin degrading system found within the cells.

Initially the effectiveness of various compounds on the insulin degrading system was examined. Haemolysate was prepared according to the method previously described (Brodal 1971) and consisted of centrifuging the blood, obtained by cardiac puncture from rats, at 2000 g for 10 min. After removal of the plasma and buffy coat the red cells were washed twice with 0·9% NaCl (saline). Haemolysates were produced by adding distilled water to the packed cells, giving a final volume of twice the original blood volume. The haemolysates were centrifuged at 18 000 *g* for 20 min and the supernatants used in the incubation assay. The incubation media consisted of 2 ml of 1:3 dilution of haemolysate, 2·9 ml of Hanks balanced salt solution containing 0·25% bovine serum albumin (HBSS-A). Insulin (25 m u), plus a trace amount of ^{125}I-labelled insulin (Amersham, U.K.), was dissolved in 1·1 ml of HBSS-A and added to the above solution to start the experiment. The incubation was carried out in a 10 ml flask maintained at 37 °C and provided with a magnetic stirrer stirring at a constant rate. Samples (400 µl) were removed at various times and added to an equal volume of 10% trichloroacetic acid (TCA). When inhibitors were used these were dissolved in the HBSS-A of the incubation media. The insulin degradation was calculated as a percentage of TCA precipitable radioactivity.

The encapsulation of insulin in erythrocytes was accomplished using the method previously described (Jenner et al 1981), which in summary consists of swelling the cells by suspending them in 0·67 × isotonic reversed Na^+/K^+ Hanks solution. The insulin to be encapsulated was dissolved in water or aqueous buffer together with a trace of ^{125}I-labelled insulin and added in portions to the cells until the tonicity of the suspension was 0·5 × the normal value. The cells were then sealed by restoring normal tonicity with 10 × isotonic Hanks solution. In the present experiments a solution of insulin 24 u ml^{-1} of water containing 35 m M tolbutamide was prepared for encapsulation. The purpose of the tolbutamide was to inhibit the degradation of insulin within the cells. By determining the radioactivity of the washed cells that had been used for the encapsulation procedure, we were able to calculate the amount of insulin in the cells. In a control experiment cells were treated with the solutions used during the encapsulation except that all solutions were isotonic. In

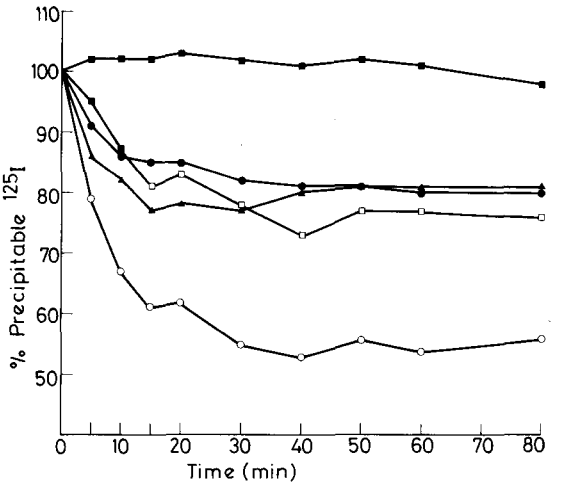

FIG. 1. Inhibition of insulin breakdown by erythrocyte haemolysates. In the absence of inhibitor only 55% of the insulin remained after 40 min (— ○ —) compared with approximately 75% with tolbutamide (20mM) (— □ —), 80% with phenformin (20 mM) (— ▲ —), 82% with DCIP (1 mM) (— ● —). When buffer was substituted for haemolysate the preparation was stable (100%) (— ■ —).

* Correspondence.

this way the amount of insulin adhering to the membrane after washing could be determined.

Fig. 1 shows the effect of three of the test compounds which inhibited the degradation of ^{125}I-labelled insulin by haemolysate. From this diagram phenformin, tolbutamide and 2,6-dichlorophenol-indophenol (DCIP) were effective inhibitors of this enzyme system allowing 80% of the insulin to survive. Bradykinin (0·1mM) and acetyl tyrosine ethyl ester (10μM) did not inhibit the enzyme. Plasma (1 cm^3) inhibited the reaction by 17%. Tolbutamide was selected as the inhibitor for the encapsulation procedure in preference to phenformin due to its superior solubility in aqueous media.

The amount of insulin encapsulated into erythrocytes was a fraction (4·8% w/w) of the extracellular insulin in solution. The maximum loading that we achieved was 57·6 m u of insulin ml^{-1} of packed cells. Cells incubated in isotonic media containing insulin and tolbutamide (controls) took up 0·5% (6·0 m u) of the insulin present. Therefore, by causing the cells to swell, it was possible to increase the loading by 51·6 m u of insulin ml^{-1} of packed cells. Previous workers (Tschesche et al 1974) have established that *N*-ethylmaleimide, *p*-chloromercuribenzoate and proteinase inhibitors isolated from the snail *Helix pomatia* inhibit the insulin degrading system in blood. Our work shows that other inhibitors, previously applicable only to insulinase found in liver extracts (Brush 1977) are equally effective against the enzyme found in erythrocytes. However, bradykinin which was found to inhibit the liver enzyme (73·5% inhibition at 0·16 nM, Brush 1977) was inactive against the red cell enzyme.

In summary we have successfully encapsulated insulin in erythrocytes but the amount incorporated in the cells (4·8%) was low compared to other drugs, e.g. methotrexate (17·4%), cyclophosphamide (15·0%), corticosteroids (16·7%) (Lewis & Raymont 1981).

We have also found that the insulin encapsulated can be stabilized by the incorporation of inhibitors in the insulin solution.

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Effect of MDL 17043, a new cardiotonic agent, on myocardial oxygen consumption

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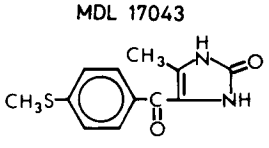
MDL 17043 (1,3-dihydro-4-methyl-5-[4-(methylthio)-benzoyl]-2*H*-imidazol-2-one) is a new non-catecholamine, non-glycoside cardiotonic agent that is presently undergoing clinical trial for the treatment of congestive heart failure. MDL 17043 has been shown to produce marked positive inotropic activity in-vivo in anaesthetized and conscious dogs (Dage et al 1982; Roebel et al 1982) and in-vitro in cat isolated atrial and papillary muscle preparations (Roebel et al 1982). The in-vivo inotropic activity is accompanied by minor increases in heart rate and brief decreases in blood pressure. MDL 17043 was able to reverse the depressant effect of pentobarbitone on the ventricular function curve in the dog heart-lung preparation as well as reverse propranolol-induced heart failure in anaesthetized dogs (Dage et al 1982). These results suggest that MDL 17043 may have beneficial effects in the clinical treatment of congestive heart failure.

* Correspondence.

MDL 17043



In the present study, we sought to determine if the marked inotropic activity produced by MDL 17043 is accompanied by substantial increases in myocardial oxygen consumption.

Materials and methods

Mongrel dogs of either sex and various weights were anaesthetized with pentobarbitone sodium (Nembutal, Abbott Laboratories) 35 mg kg^{-1} via the cephalic vein. After tracheostomy, respiration was maintained with a