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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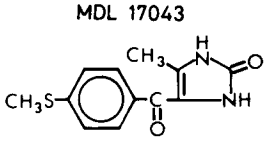
MDL 17043 (1,3-dihydro-4-methyl-5-[4-(methylthio)-benzoyl]-2*H*-imidazol-2-one) is a new non-catecholamine, non-glycoside cardiotonic agent that is presently undergoing clinical trial for the treatment of congestive heart failure. MDL 17043 has been shown to produce marked positive inotropic activity 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

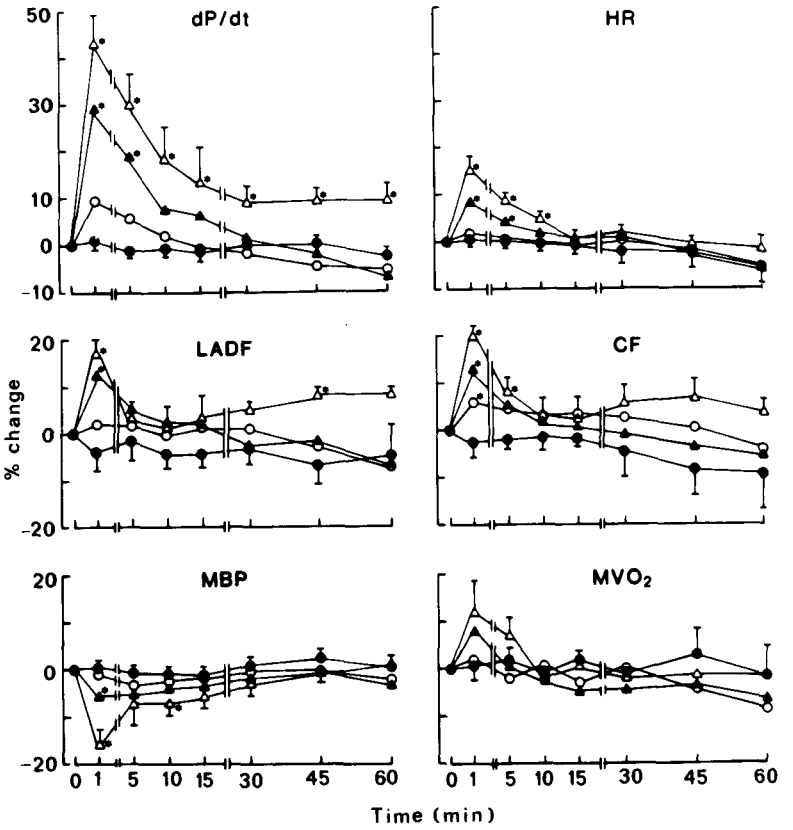


FIG. 1. Effect of MDL 17043 on cardiac contractility (dP/dt), heart rate (HR), left anterior descendens blood flow (LADF), circumflex blood flow (CF), mean blood pressure (MBP), and myocardial oxygen consumption (MVO_2) in the anaesthetized dog. An intravenous dose of 0·1 mg kg^{-1} (○), 0·3 mg kg^{-1} (▲) or 1 mg kg^{-1} (△) was given to 5 dogs. Vehicle (●) in an amount equivalent to the 1 mg kg^{-1} dose of drug was given to 5 dogs. The range of mean pretreatment values for the 4 groups was: dP/dt, from 1676 ± 63 to 2153 ± 92 mm Hg s^{-1}; heart rate, from 160 ± 14 to 164 ± 8 beats min^{-1}; left anterior descendens flow, from 30·4 ± 4·9 to 34·5 ± 5·1 ml min^{-1}; circumflex flow, from 32·2 ± 7·5 to 42·9 ± 10·5 ml min^{-1}; mean blood pressure, from 102 ± 6 to 115 ± 5 mm Hg; and myocardial oxygen consumption, from 6·9 ± 1·4 to 7·7 ± 1·2 ml O_2 min^{-1}. Values shown are means with or without standard errors. * = significant effect compared with the vehicle group by one-tailed Dunnett's test ($P < 0.05$).

Palmer respirator. The left femoral vein was cannulated for intravenous drug administration. The right femoral artery was cannulated and the cannula attached to a Statham P23Ac pressure transducer to measure arterial blood pressure. The left femoral artery was cannulated for subsequent withdrawal of arterial blood samples for oxygen content analysis. The lead II electrocardiogram (e.c.g.) and heart rate were recorded using needle electrodes and a Grass Model 7P4 ECG Tachograph preamplifier. After a thoracotomy at the left fifth intercostal space, a pressure transducer (Konigsberg P6.5) was implanted into the left ventricle of the heart through a stab incision at the apex. A differentiator (Grass P20) was used to measure dP/dt, an index of cardiac contractility. Left anterior descendens flow (LADF) and circumflex flow (CF) were measured using Statham blood flow transducers of appropriate size placed around the left anterior descending branch of the left coronary artery and the circumflex branch of the left coronary artery, respectively. All recordings were made on a Grass polygraph (Model 7D).

An Elecath angiographic catheter was placed in the coronary sinus via an incision in the right atrial appendage. The catheter was used for the subsequent withdrawal of coronary sinus blood for oxygen content analysis. Simultaneously withdrawn arterial and coronary sinus blood samples were analysed in duplicate for oxygen content using a LEX O_2 Con-TL (Lexington Instrument Corp., Waltham, Mass.). Myocardial oxygen consumption (MVO_2, in ml O_2 min^{-1}) was calculated using the formula: MVO_2 = coronary blood flow × A–V difference ÷ 100; where coronary blood flow (in ml min^{-1}) was equal to the sum of LADF and CF and A–V difference was equal to the difference in oxygen content (in vol %) of the arterial and coronary sinus blood samples. Arterial and coronary sinus blood

samples were withdrawn pre-dose and at various times after dosing.

Animals were dosed intravenously with either vehicle (volume equal to largest volume of drug solution given) or one of 3 doses of MDL 17043 (0·1, 0·3 or 1 mg kg^{-1}). MDL 17043 was dissolved in 0·12 ml of 1M sodium hydroxide for each 10 mg of drug and diluted to the appropriate volume with Sorensen's phosphate buffer. The final pH of the MDL 17043 and vehicle solutions ranged from 11·9–12·2. All solutions were prepared immediately before use.

A one-tailed Dunnett's test was used to compare results obtained with the vehicle vs the MDL 17043-treated groups (Winer 1971).

Results

The cardiovascular effects of MDL 17043 or vehicle administered intravenously to anaesthetized dogs can be seen in Fig. 1. MDL 17043 produced dose-related increases in cardiac contractility (dP/dt), heart rate, and coronary artery blood flow, and dose-related decreases in mean arterial pressure. There were no significant changes in myocardial oxygen consumption seen with MDL 17043.

The statistically significant increase in dP/dt produced by the 0·3 and 1 mg kg^{-1} doses of MDL 17043 peaked at 1 min after dosing and remained significantly elevated for 5 min and 60 min, respectively. Likewise, the heart rate increases produced by MDL 17043 also peaked at 1 min. Heart rate remained significantly elevated for 5 min after the 0·3 mg kg^{-1} dose and for only 10 min after the 1 mg kg^{-1} dose. Coronary blood flow increases and mean arterial blood pressure decreases were modest and relatively short-lasting.

In spite of the significant changes in dP/dt and heart rate, both of which should result in increased oxygen consumption, there were no significant changes seen in myocardial oxygen consumption. There were, however, slight transient increases in oxygen consumption which coincided with the peak increase in cardiac contractility and heart rate produced by MDL 17043.

Discussion

The haemodynamic effects observed with MDL 17043 in these experiments are qualitatively similar to those reported previously (Dage et al 1982; Roebel et al 1982); namely, marked positive inotropic activity accompanied by minor increases in heart rate and brief decreases in blood pressure. In addition, this study shows that inotropic doses of MDL 17043 produced marked increases in cardiac contractility without significantly increasing myocardial oxygen consumption. The known principal determinants of myocardial oxygen consumption are cardiac contractility, heart rate and ventricular wall tension (Mason et al 1976). Our finding that MDL 17043 increased both cardiac contractility and heart rate without increasing myocardial oxygen consumption suggests that MDL 17043 lowered ventricular wall tension. The hypotensive effect of MDL 17043 would certainly contribute to such a decrease in wall tension. Additionally, ventricular volume may also have been decreased by MDL 17043 since in an earlier study the compound was shown to increase cardiac contractile force without increasing stroke volume, suggesting that MDL 17043 increases venous capacitance (Dage et al 1982). Therefore, the lack of effect of MDL 17043 on myocardial oxygen consumption in these experiments resulted from the probable decrease in ventricular wall tension decreasing myocardial oxygen demand nearly as much as the positive inotropic and chronotropic effects of MDL 17043 increased it.

Although positive inotropic doses of MDL 17043 did not significantly change myocardial oxygen consumption in the normal heart, it may decrease myocardial oxygen consumption in the failing heart, since this is the case with the digitalis glycosides. In the normally functioning heart, digitalis increases myocardial oxygen consumption (Covell et al 1966; Coleman 1967). However, when a digitalis glycoside is administered to a failing heart preparation, myocardial oxygen consumption decreases, since the diminished oxygen requirement brought about by a decrease in wall tension is greater than the increase in oxygen consumption caused by the increase in contractility (Covell et al 1966; Braunwald 1971). Thus, it would be of interest to compare the lack of effect of MDL 17043 on oxygen consumption in the present experiments with its effect in animals with either drug-induced or surgically-induced heart failure.

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