科技资料

Acute Heart Failure in Intensive Care: A New Approach

Acute Heart Failure in Intensive Care: A New Approach

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Drug Dosage

The authors and the publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accord with current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new and/or infrequently employed drug.

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Introduction

The human heart, in common with many organs in the body, has an amazing reserve capacity. This enables it to withstand all manner of abuses, including cardiac surgery. Just occasionally, however, the cardiac surgical team may be faced with the challenge of treating a patient whose heart is devoid of this reserve capacity and can barely sustain the vital circulation. In this situation a logical and rational approach to therapy requires a comprehensive understanding of the pathophysiology of cardiac failure.

In the human heart, the β-adrenergic receptor/adenylate cyclase pathway is the primary mechanism which can be manipulated to improve cardiac performance. Most of the inotropes in current use act through the β-adrenoceptor and sometimes to a lesser degree on the inhibitory coupling G protein. Unfortunately, as cardiac failure progresses, β-adrenoceptor function may be substantially reduced. In these circumstances, the therapeutic strategy should be to find an alternative pathway to increase cyclic AMP and intracellular calcium, and at the same time to prevent further downgrading of the β-adrenoceptors.

The papers collected here were presented at the satellite symposium, entitled 'Acute Heart Failure in Intensive Care: a New Approach', at the 1989 spring meeting of the Association of Cardiothoracic Anaesthetists of Great Britain and Ireland. They report on the current experience with enoximone, which belongs to a new class of drugs that increase the level of cyclic AMP in myocardial cells by selectively inhibiting the phosphodiesterase responsible for the inactivation of cyclic AMP.

In the myocardium, cyclic AMP increases the concentration of intracellular calcium via the protein kinase system: the uptake of calcium into the sarcoplasmic reticulum is facilitated, and the flux of calcium through the sarcolemmal channels is increased. This process not only enhances contraction but also, perhaps more importantly, aids relaxation of the heart. Cyclic AMP also brings about relaxation in the smooth muscle of blood vessels, thereby reducing systemic and pulmonary vascular resistance.

This combined inotropic and vasodilatory action of enoximone, mediated by a mechanism which bypasses the β-adrenoceptors, improves cardiac function significantly in patients with heart failure, without increasing oxygen demand. It may have a particularly important role to play in patients who are refractory to treatment with catecholamines.

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Pharmacology and Pharmacokinetics of Enoximone

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Key Words. Congestive heart failure · Enoximone · Enoximone sulphoxide · Pharmacology · Metabolism

Abstract. Enoximone is an inotropic vasodilating agent. Its principal effects are positive inotropism and vasodilation, which are not accompanied by changes in myocardial oxygen consumption. An inotropic dose of enoximone increases the level of cyclic AMP in the isolated, blood-perfused dog papillary muscle owing to its selective inhibition of the one isoform of cyclic AMP phosphodiesterase from the dog heart that is inhibited by cyclic GMP. Studies on the metabolism and pharmacokinetic profile of enoximone have been carried out in the rat, dog and monkey. Enoximone is metabolized mainly by oxidation to enoximone sulphoxide in all species studied, and this is reversible. In congestive heart failure patients, approximately 74% of a rapidly administered intravenous dose of enoximone is excreted in a 24-hour urine collection as the sulphoxide metabolite; only about 0.49% is recoverable as intact drug. Enoximone sulphoxide has the same inotropic and vasodilator activities as enoximone but is 0.13–0.14 times as potent and has a 13 times longer duration of inotropic action in the dog. It is suggested that the metabolite may contribute to some of the effects that follow enoximone administration.

Introduction

Enoximone is a new cardiotonic agent, for use in the treatment of acute congestive heart failure, which has a dual action with both positive inotropic and vasodilator activities. It is active by intravenous and oral routes but is presently available only as an injection. The animal pharmacology, metabolism and pharmacokinetics pertinent to the clinical development of enoximone are reviewed.

Enoximone belongs to a new class of cardio-active compounds, referred to as imidazolones (4-aroylimidazol-2-ones; (fig. 1) [1], which were synthesized for the purpose of finding a cardiotonic agent with a wider therapeutic index than that exhibited by the

digitalis glycosides. It is not related structurally to either the catecholamines or the digitalis glycosides.

Direct positive inotropic, positive chronotropic and vasodilatory properties of enoximone were first demonstrated in anaesthetized dogs (fig. 2) [2]. The principal effects of intravenous injections, 0.1, 0.3 and 1 mg/kg, were dose-related increases in cardiac contractile force, accompanied by brief decreases in systemic blood pressure and minor increases in heart rate. These effects were not associated with an increase in myocardial oxygen consumption [3]. Further studies revealed that these were direct effects of enoximone, as they were not attenuated by reserpinization, carotid sinus denervation or bilateral vagotomy [4]. Intravenous infusion of enoximone, 0.3 and 3 mg/kg, produced sustained decreases in systemic blood pressure without increasing cardiac output or heart rate, indicating that it decreased vascular resistance [2]. The vasodilating effects were confirmed in dog hind limb preparations perfused with a constant flow of blood: injections of enoximone, 0.3 and 3 mg/kg i.v., produced a dose-related decrease in hind limb perfusion pressure (fig. 3) [4, 5]. The vasodilation in the hind limb was not attenuated by surgical sympathectomy, indicating that it was directly mediated. Enoximone was a more effective positive inotropic agent than vasodilator in the isolated, blood-perfused dog papillary muscle preparation [6].

The depressant effects of sodium pentobarbital on cardiac function could be reversed by enoximone in the dog heart-lung preparation (fig. 4) [2]. Cardiac function was improved by enoximone in anaesthetized dogs and dog heart-lung preparations pretreated with myocardial depressant quantities of propranolol. The improvement in car-

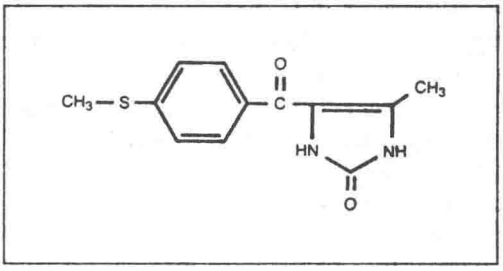


Fig. 1. Structure of enoximone: 1,3-dihydro-4-methyl-5-[4-(methylthio)benzoyl]-2H-imidazol-2-one.

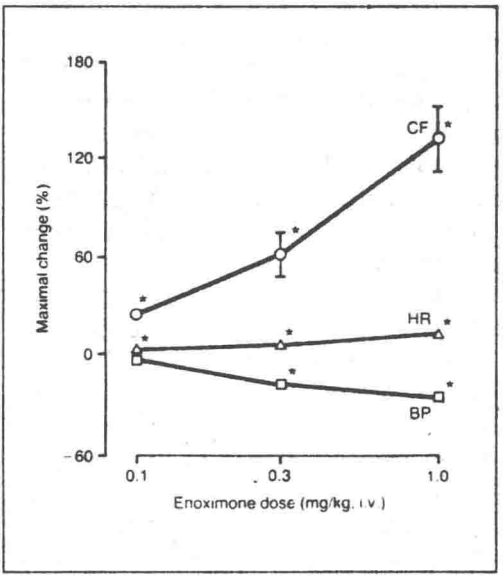


Fig. 2. Effects of intravenous enoximone on cardiac contractile force (CF), heart rate (HR) and mean systemic blood pressure (BP) in anaesthetized dogs. Values are means ± SEM of the maximum effect observed in 10 min; n = 5. * $p < 0.05$: significant effect (t test). Pretreatment values for the groups treated with 0.1, 0.3 and 1.0 mg/kg were 134 ± 6, 130 ± 12 and 124 ± 8 g (CF); 156 ± 4, 140 ± 12 and 130 ± 5 beats/min (HR), and 110 ± 10, 106 ± 5 and 107 ± 6 mm Hg (BP), respectively. Reproduced with permission from Dage et al. [2].

diac function produced by a moderately inotropic dose of enoximone, 0.3 mg/kg i.v., was manifested primarily as a decrease in left atrial pressure (fig. 5) [2]. Stroke volume did not change, which suggests that enoximone increased venous capacitance and/or left ventricular compliance. Thus, along with its positive inotropic and afterload-reducing properties, enoximone may also reduce preload. These properties of enoximone suggested therapeutic value in patients with congestive heart failure.

The effects of enoximone on cardiac and smooth muscle were studied further in several in vitro preparations, though its very low aqueous solubility (0.014 mg/ml) at physiological pH has made interpretation of its potency difficult. Cumulative concentrations of enoximone ranging from 10^{-5} to 10^{-3} mol/l produced dose-dependent increases in contractile force in both papillary muscle and left atrial strip preparations from cats or guinea pigs; atrial rate increased to a lesser extent. By contrast, no significant inotropic chronotropic specificity was demonstrated with isoprenaline (fig. 6) [4]. The inotropic potency of enoximone was approximately 0.0001–0.0004 times that of isoprenaline, whereas the chronotropic relative potency was much less (6×10^{-6}; table 1). The time to peak tension, relaxation time and total duration of contraction were shortened at these concentrations of enoximone in dog ventricular trabeculae [7]. Enoximone, 10^{-5} to 10^{-3} mol/l, also produced dose-related relaxations of carbachol-contracted guinea pig trachea and was 0.0005 times as potent as isoprenaline in this preparation. The inotropic effectiveness of an identical range of blood concentrations of enoximone was demonstrated in the dog heart-lung preparation [2].

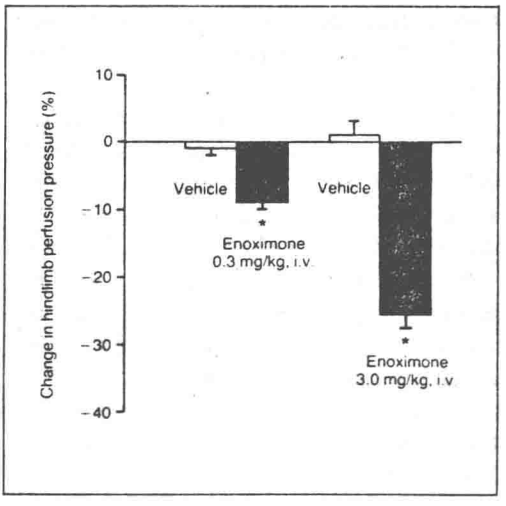
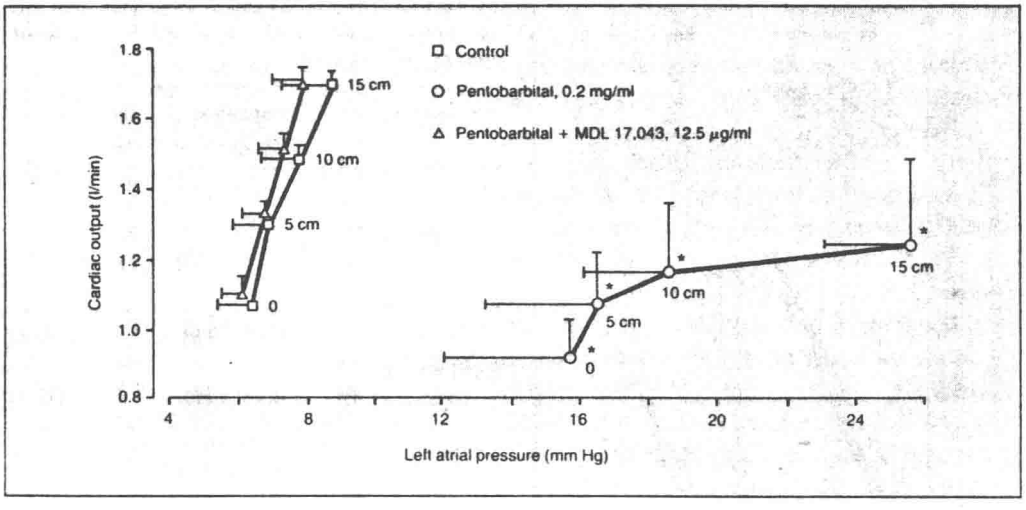


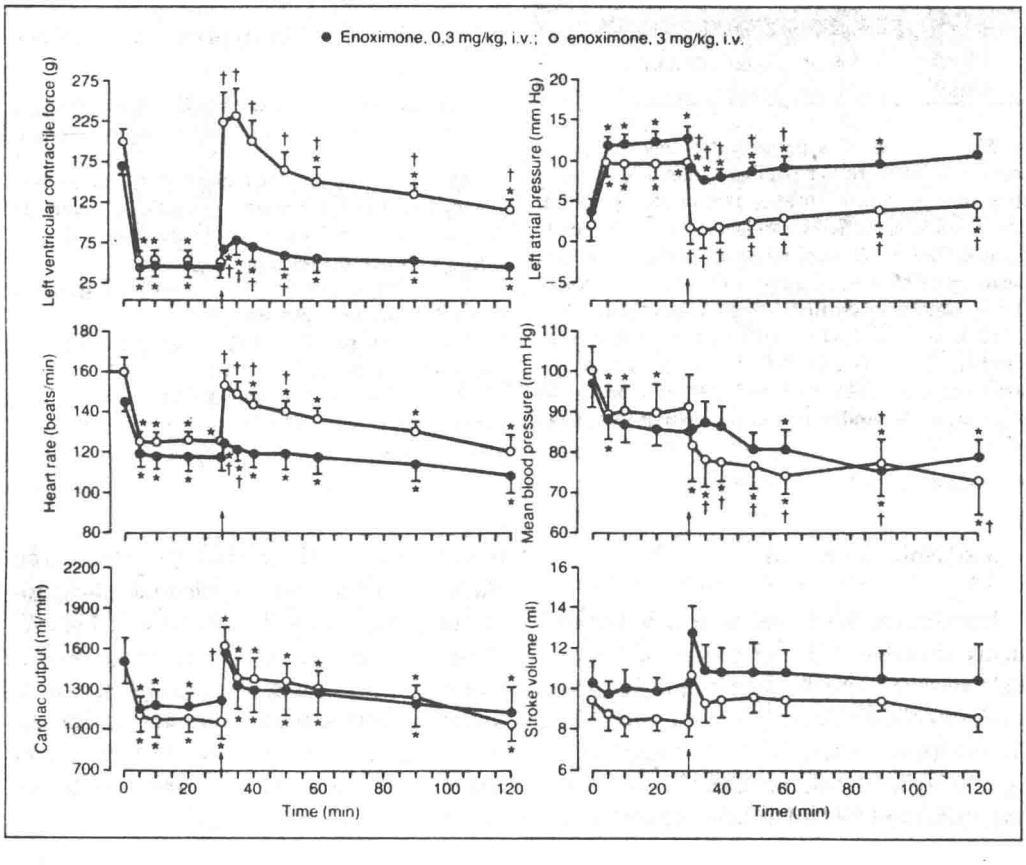
Fig. 3. Effect of enoximone (0.3 and 3 mg/kg i.v.) on hind limb perfusion pressure in 6 anaesthetized dogs. Means ± SEM of the maximum effects are shown. * $p < 0.05$: Significant difference from vehicle (t test). Reproduced with permission from Dage et al. [5].

Fig. 4. Effects of pentobarbital and enoximone on left ventricular function in the dog heart-lung preparation. Values were obtained by raising the venous reservoir 5, 10, and 15 cm above the primary value (0) and recording the resultant left atrial pressure and cardiac output. Mean aortic blood pressure was maintained at approximately 89.9 ± 0.6 mm Hg throughout. Data are for 6 preparations determined before and 5–10 min after sequential additions of pentobarbital or enoximone. * $p < 0.05$: significant difference from control values (multivariate analysis of variance). Reproduced with permission from Dage et al. [2].

Fig. 5. Effects of enoximone in anaesthetized dogs infused with a myocardial depressant dose of propranolol. Propranolol, 4 mg/kg i.v., was injected at time zero, followed immediately by a constant infusion, 0.18 mg/kg/min i.v., for 120 min. This produced many of the haemodynamic characteristics of heart failure. Enoximone, 0.3 mg/kg i.v. or 3 mg/kg i.v., was given at 30 min (arrow). Values are means ± SEM; $n = 6$. * $p < 0.05$, + $p < 0.05$: significant effect compared to zero time value and 30-min value (pre-enoximone), respectively, (t test). Reproduced with permission from Dage et al. [2].


Control
Pentobarbital, 0.2 mg/ml
Pentobarbital + MDL 17,043, 12.5 µg/ml
Cardiac output (l/min)
Left atrial pressure (mm Hg)
15 cm
10 cm
5 cm
0

4


Enoximone, 0.3 mg/kg, i.v.; enoximone, 3 mg/kg, i.v.
Left ventricular contractile force (g)
Left atrial pressure (mm Hg)
Heart rate (beats/min)
Mean blood pressure (mm Hg)
Cardiac output (ml/min)
Stroke volume (ml)
Time (min)
Time (min)

5

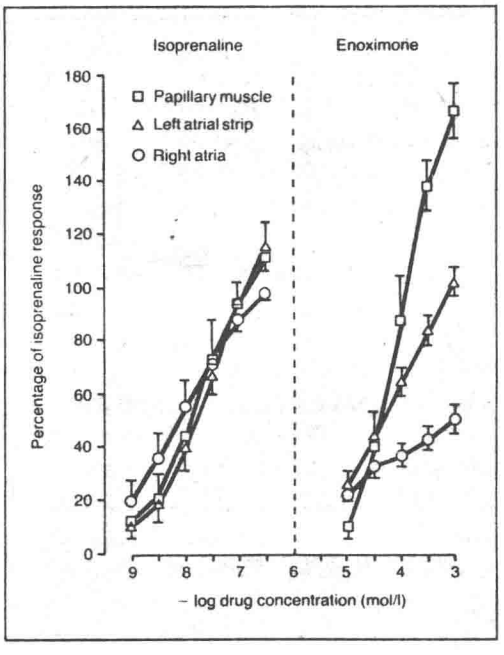


Fig. 6. Effects of isoprenaline and enoximone on contractile force of cat papillary muscle, left atrial strip and spontaneous beating rate of cat right atria. The stimulatory effects produced by isoprenaline, 3.16×10^{-7} mol/l, were taken as 100%. Values are means ± SEM; n = 5. Control values for enoximone- and isoproterenol-treated tissues, respectively, were 1,110 ± 461 and 1,540 ± 689 mg tension (papillary muscle), 430 ± 71 and 395 ± 101 mg tension (left atrial strip) and 105 ± 8 and 100 ± 4 beats/min (right atria). Reproduced with permission from Roebel et al. [4].

Table 1. Relative potencies of isoprenaline and enoximone on force and rate of cardiac contraction and tracheal relaxation

Preparation	Isoprenaline ($-\log EC_{50}$)	Enoximone ($-\log EC_{50}$)
Cat		
Left atrial strips (force)[1]	7.80 ± 0.14 (5)	4.35 ± 0.19 (5)
Papillary muscle (force)[1]	7.84 ± 0.26 (5)	4.34 ± 0.08 (5)
Right atrium (rate)	8.32 ± 0.40 (5)	< 3.0 (5)
Guinea pig		
Left atrial strips (force)[2]	8.28 ± 0.08 (8)	4.22 ± 0.16 (6)
Atria (rate)	8.25 ± 0.16 (5)	< 3.0 (6)
Tracheal strips (relaxation)[3]	7.91 ± 0.11 (7)	4.60 ± 0.12 (6)

Means ± SEM of – log molar concentrations producing half-maximal response ($-\log EC_{50}$). Figures in parentheses indicate numbers of experiments. Reproduced with permission from Dage et al. [5]

[1] Electrically paced at 0.2 Hz, 5 ms pulse duration and 1.2 times threshold voltage.

[2] Electrically paced at 3 Hz, 5 ms pulse duration and 1.4 times threshold voltage.

[3] Tissues were contracted with 3.16×10^{-7} mol/l carbacholine.

Mechanism of Action

Enoximone was found to be a potent and highly selective inhibitor of one of the several types of cyclic nucleotide phosphodiesterases isolated from dog, bovine and guinea pig ventricles, whereas the standard inhibitors, 3-isobutyl-1-methylxanthine and papaverine, inhibited all of these phosphodiesterases non-selectively [8–10] (table 2). The classification of cyclic nucleotide phosphodiesterases [11] is shown in table 3. Types I, II and IV enzymes are found in dog, bovine and guinea pig hearts. The type IV enzyme is found in cardiac tissue from human cardiomyopathic patients [12]. Recently, it was reported that there are two isoforms of the type IV, or high-affinity cyclic AMP phos-

Table 2. Comparison of the inhibitory potencies of enoximone, and 3-isobutyl-1-methylxanthine (IBMX) on the different cyclic nucleotide phosphodiesterases (PDE) from the dog heart

Drug	PDE-I	PDE-II	PDE-IV
Enoximone, μmol/l	> 200	> 100	7.6
IBMX, μmol/l	18.6	7.5	7.5

Concentrations producing 50% inhibition of the hydrolysis of AMP by the PDE were measured. PDE-I data obtained in the absence of calmodulin (1 Sigma unit/13.75–12.5 μg protein) and calcium (10 μmol/l), but addition of these to the test system made no difference. Reproduced with permission from Dage et al. [5].

Table 3. Cyclic nucleotide phosphodiesterases: nomenclature recommendation[1]

Type I	Calmodulin-sensitive cyclic nucleotide phosphodiesterase. This enzyme hydrolyses both cyclic AMP and cyclic GMP, and its activity is enhanced by Ca^{2+}-calmodulin.
Type II	Cyclic-GMP-sensitive cyclic nucleotide phosphodiesterase. This enzyme also hydrolyses both cyclic AMP and cyclic GMP, but its cyclic-AMP-hydrolytic activity is enhanced by cyclic GMP.
Type III	Rhodopsin-sensitive cyclic GMP phosphodiesterase.
Type IV	Cyclic AMP phosphodiesterase. This enzyme is relatively specific for cyclic AMP and had been referred to as the low-K_m or high-affinity cyclic AMP phosphodiesterase. It has also been referred to as phosphodiesterase III based on its order of elution from a chromatographic column. It consists of two subtypes [10]: (a) cyclic-GMP-inhibitable form; (b) cyclic-GMP-noninhibitable form (suggested type V).

[1] Types I–IV recommended by Appleman et al. [11].

phodiesterase, and that enoximone inhibits only one of these [10]. The enoximone-sensitive isoform of type IV phosphodiesterase is characteristically inhibited by cyclic GMP but not by rolipram, another phosphodiesterase inhibitor [9, 13]. By contrast, the other isoform of type IV phosphodiesterase is both enoximone and cyclic GMP insensitive, but it is inhibited by rolipram [13]. Enoximone did not inhibit either the calmodulin-sensitive cyclic nucleotide phosphodiesterase (type I) or the cyclic-GMP-stimulated cyclic nucleotide phosphodiesterase (type II) at practical concentrations (100–200 μmol/l) [5].

The type of inhibition produced by enoximone was, at first, controversial, because early studies were carried out with mixtures of the type IV isoforms. In these early studies, inhibition by enoximone was reported by some to be partially competitive and by others to be competitive [8, 9]. However, with the purified enzyme isolated from the bovine ventricle by precipitation with monoclonal antibodies, inhibition by enoximone was competitive [10]. Furthermore, in the presence of rolipram, the inhibition of type IV phosphodiesterase by enoximone is competitive (fig. 7) [13].

Specific inhibition of type IV phosphodiesterase seems to have a role in the positive inotropic action of enoximone; this agent was reported to produce an increase in cyclic AMP in the dog ventricular muscle during the peak of its inotropic effect (fig. 8) [6, 7]. A cyclic-AMP-dependent mechanism of inotropism for enoximone is supported by a report showing inhibition of the positive inotropic effect of enoximone in isolated dog trabeculae by carbachol, an agent known to attenuate adenylate cyclase activity and cyclic AMP formation [7].

Studies in vivo and in vitro with appropriate antagonists have indicated that the cardiac and vasodilator effects of enoximone are not mediated by stimulation of either α- or β-adrenergic receptors or H_1- or H_2-histaminergic receptors. Furthermore, the vasodilator effect does not involve stimulation of muscarinic receptors or altered prostanoid formation [2, 4]. Enoximone, 1 mmol/l, does not alter the calcium sensitivity of chemically skinned (glycerinated) myocardial fibers from pig hearts [14], and it did not stimulate the Mg^{2+}-ATPase of dog cardiac myofibrils [Kariya and Dage, unpubl. data]. Biochemical studies have demonstrated that enoximone did not directly affect oxalate-supported Ca^{2+} uptake into dog cardiac sarcoplasmic reticulum vesicles, and at practicable levels (50–100 μmol/l) it did not directly alter the activity of the Ca^{2+}-ATPase or adenyl cyclase from dog hearts [8]. However, enoximone, 100 μmol/l, causes a 54% inhibition of the Na^+-K^+-ATPase from the dog kidney (table 4) [8]. Although this enoximone concentration corresponds with the upper part of the dose-response curve for inotropism [2], it is unlikely that Na^+-K^+-ATPase inhibition plays a role in the inotropic action of enoximone, as there is no interaction between large inotropic doses of enoximone and ouabain in anaesthetized dogs [4].

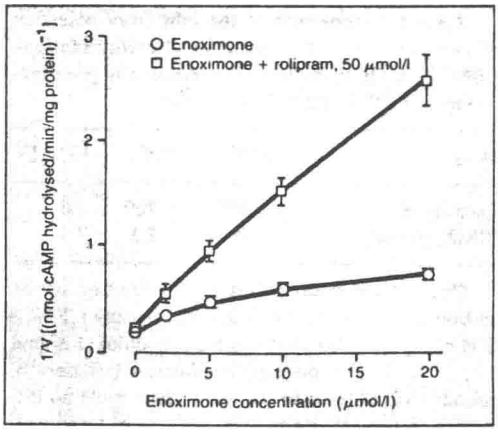


Fig. 7. Dixon plots of dog cardiac high-affinity cyclic AMP phosphodiesterase inhibition by enoximone and by enoximone + rolipram. Rolipram was used to inhibit the enoximone-insensitive cyclic AMP phosphodiesterase. Values are means ± SEM; n = 4 different enzyme preparations. Reproduced with permission from Kariya and Dage [13].

Table 4. Effect of enoximone on Na^+-K^+-ATPase from dog kidneys

Concentration, μmol/l	Inhibition, %
10	0
30	2.3
100	54.0

Results are means of 3 or 4 assays. Average specific activity of the enzyme was 56.4 μmol P_i released/mg protein/h. Reproduced, in part, with permission from Kariya et al. [8].

Drug Interaction Studies

Interactions between enoximone and selected cardiovascular drugs used in the treatment of conditions associated with congestive heart failure have been studied [2, 4]. Enoximone reversed the depressant effects of verapamil and pentobarbital on cardiac function in the dog heart-lung preparation (fig. 9). Moreover, it also reversed the atrioventricular block produced by verapamil [Dage, unpubl. data]. In anaesthetized dogs, the cardiovascular effects of enoximone were unaltered by α- and β-adrenergic recep-

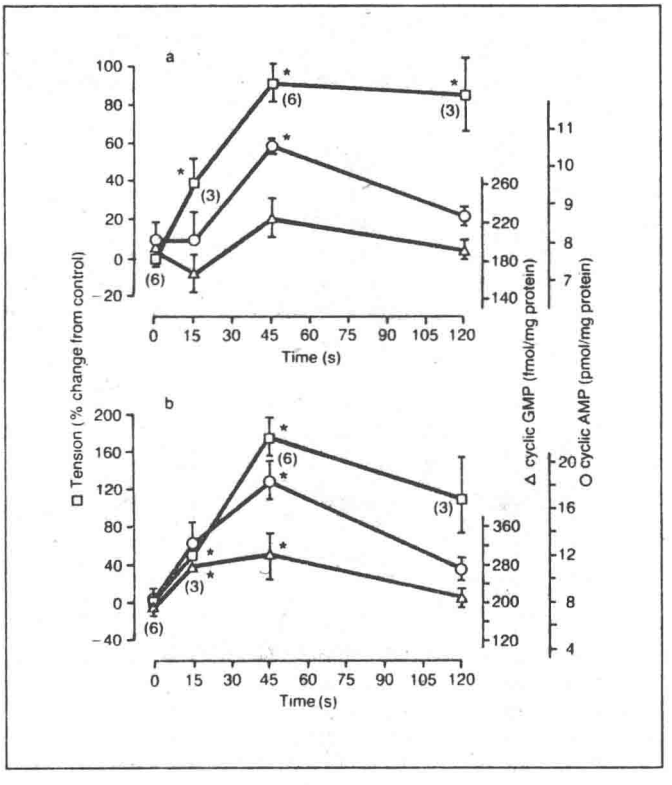


Fig. 8. Time course of changes in developed tension, cyclic AMP and cyclic GMP produced by an infusion of enoximone, 1 mg/15 s (**a**) or 3-isobutyl-1-methylxanthine (**b**) into blood-perfused dog papillary muscle. Time is seconds after onset of inotropic response. Values are means ± SEM; numbers are given in parentheses. * $p < 0.05$: significant difference compared with respective controls (analysis of variance or t test). Reproduced with permission from Hsieh et al. [6].

tor antagonistic doses of phentolamine and propranolol [4]. However, the vasodilatory responses produced by intravenous enoximone were enhanced after pretreatment with phenoxybenzamine [4]. Pretreatment with nitroglycerin, 3 μg/kg/min i.v., or furosemide, 5 mg/kg i.v., did not alter the cardiovascular effects of enoximone, 0.1–1 mg/kg i.v., in anaesthetized dogs [4]. Pretreatment with hydralazine, 0.3 mg/kg i.v., had no effect on the enoximone-mediated effects on blood pressure and heart rate but attenuated the positive inotropic effect produced by administration of enoximone, 1 mg/kg [4].

Ouabain, 1 μg/kg/min i.v., did not alter the inotropic or chronotropic effects of enoximone (1 mg/kg i.v.) but attenuated the decrease in blood pressure brought about by this agent [4]. Enoximone, 1 mg/kg i.v., did not cause arrhythmias nor did it alter arrhythmias caused by ouabain in anaesthetized dogs.

Metabolism

The metabolism and pharmacokinetic profile of enoximone was studied in the rat, dog and monkey. In these species, metabolism of enoximone appears qualitatively similar to that in man. Enoximone is metabolized mainly by oxidation to enoximone

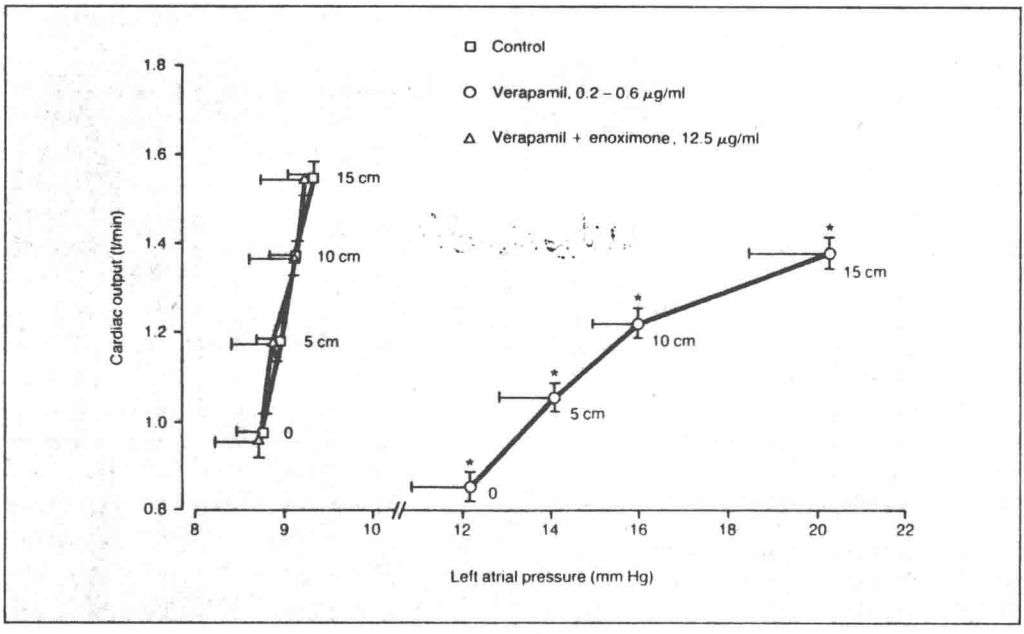


Fig. 9. Effects of verapamil and enoximone on left ventricular function in the dog heart-lung preparation. Left ventricular function curves were produced by raising the venous reservoir 5, 10 and 15 cm above the primary value (0) and measuring the resultant left atrial pressure and cardiac output. Mean aortic blood pressure was kept constant throughout, and the preparations were paced at 2.8 Hz. Data are means ± SEM for 6 preparations determined before and approximately 5–10 min after sequential additions of verapamil or enoximone. * $p < 0.05$: significant difference from control values (multivariate analysis of variance). Reproduced with permission from Dage et al. [5].

sulphoxide in all species studied. This metabolism is reversible in animals (and, presumably, in man; fig. 10) [15–18]. In congestive heart failure patients, about 74% of a rapidly administered intravenous dose of enoximone, ranging from 0.5 to 3.0 mg/kg, is excreted in the 24-hour urine collection as the sulphoxide metabolite [18]; only about 0.49% of the dose is recoverable in the urine as intact drug, and a similar quantity is recoverable as the sulphone metabolite. The interconversion of enoximone and enoximone sulphoxide was demonstrated in anaesthetized dogs, where intravenous injections of 3 mg/kg of either agent gave rise to the other in the plasma (fig. 11). In anaesthetized dogs, the sulphoxide metabolite administered by intravenous injection was found to be 0.14 times as potent as enoximone in its inotropic action, while the duration of inotropic action was 13 times longer (fig. 12). Similarly, in the isolated blood-perfused dog papillary muscle, the sulphoxide metabolite was 0.13 times as potent as enoximone inotropically and produced vasodilation as well. In congestive heart failure patients receiving enoximone, 0.5 mg/kg/min, rapidly by intravenous injection followed by

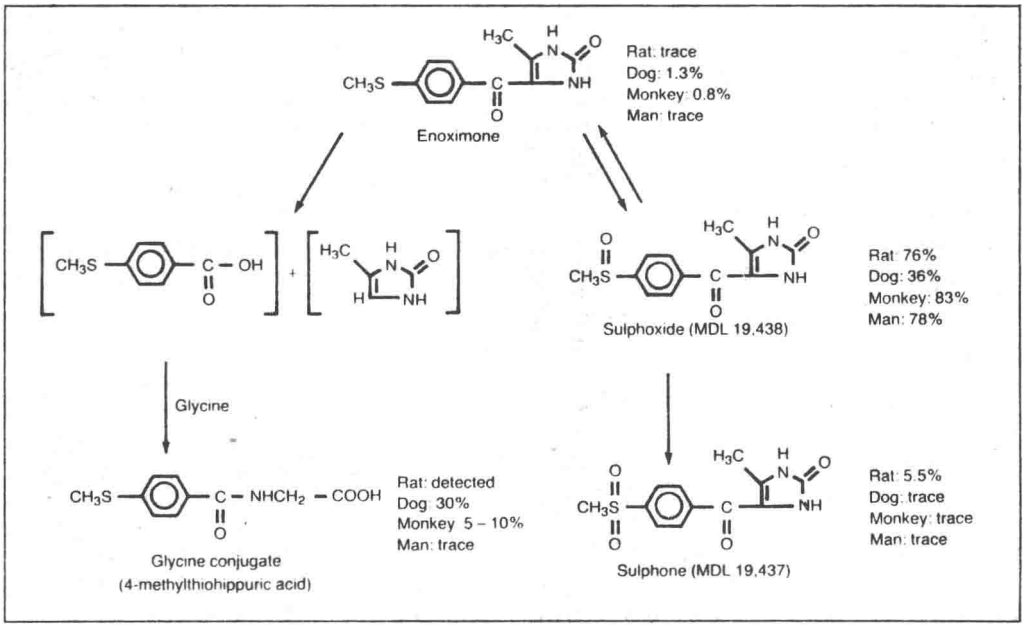


Fig. 10. Known metabolic pathways for enoximone (bracketed compounds are postulated intermediates). Percentage values are average urinary recoveries. Rat data were obtained from 24-hour urine collections after intravenous doses, 5 mg/kg, to 5 male Charles River Sprague-Dawley (CD) rats. Dog data were obtained from 24-hour urine collections after intravenous doses, 10 mg/kg, to 3 male beagle dogs. Monkey data were obtained from 24-hour urine collections after intravenous doses, 15 mg/kg, to 3 male cynomolgus monkeys. Human data were obtained from 8-hour urine collections after oral solution doses, 2 mg/kg, to 8 healthy male subjects who had fasted overnight. Chemical entities were identified and quantitated using sensitive and specific high-performance liquid chromatography and gas chromatography-mass spectrometry. Reproduced with permission from Okerholm et al. [18].

infusion, 5 μg/kg/min, the area under the curve of sulphoxide plasma concentration and time was approximately 3.2 times greater than that for enoximone, and this ratio can become greater under conditions of either lower or higher infusion rates and renal disease (fig. 13). The steady-state volume of distribution in this study was approximately 1.6 l/kg, and the terminal elimination half-life was approximately 4.3 h. Some contribution by the sulphoxide metabolite to the cardiovascular responses of enoximone is conceivable under certain circumstances; however, the degree of contribution has not been established.

Conclusion

Enoximone is an agent with both positive inotropic and vasodilatory properties, that can produce an improvement in cardiac function, in vivo, without an increase in myocardial oxygen consumption. The ac-

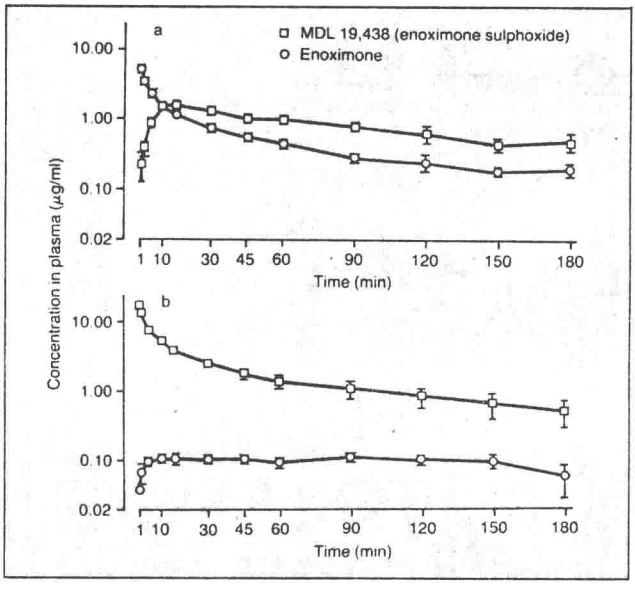


Fig. 11. Plasma levels of enoximone and enoximone sulphoxide (MDL 19,438) in anaesthetized dogs after a dose of enoximone, 3 mg/kg i.v. (**a**), or enoximone sulphoxide, 3 mg/kg i.v. (**b**). Values are means ± SEM; n = 5.

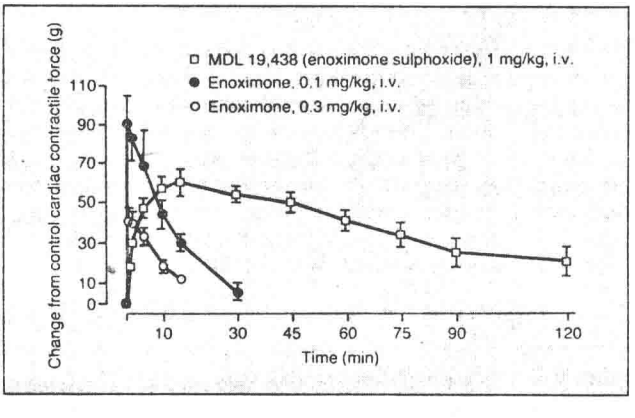


Fig. 12. Comparison of the effects of enoximone and enoximone sulphoxide (MDL 19,438) on cardiac contractile force measured with a strain gauge arch-sutured to the left ventricle of anaesthetized dogs. Values are means ± SEM; n = 7.

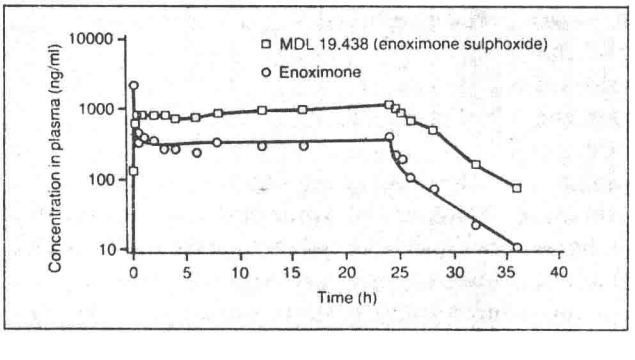


Fig. 13. Plasma concentration-time profile of enoximone and its sulphoxide metabolite resulting from administration of a slow bolus of enoximone, 0.5 mg/kg i.v., followed by a constant rate infusion, 5 μg/kg/min i.v., to a congestive heart failure patient. The lines associated with the enoximone data points are fitted curves.

of cyclic AMP and hence activation of cyclic-AMP-dependent protein kinase [3]. Phosphorylation of calcium channels, phospholamban and contractile proteins, catalysed by protein kinase, leads to an increase in the rate and force of contraction [4]. Phosphodiesterase IV has a high affinity for cyclic AMP and induces hydrolysis, limiting its activity. Consequently, phosphodiesterase IV inhibition enhances inotropic responses to β-AR stimulation [5].

The primary objective of this study was to determine whether phosphodiesterase IV inhibition is able to regulate differentially β_1-AR- and β_2-AR-mediated inotropic effects. This question arose from previous studies of selective β_1-AR and β_2-AR stimulation in isolated strips of human atrial myocardium, where following chronic treatment with β_1-selective antagonists (atenolol or metoprolol), the responsiveness of atrial myocardium to β_2-AR stimulation was selectively enhanced [2]. The aim was to ascertain whether, in patients chronically treated with β_1-selective antagonists, a selective increase in β_2-AR responsiveness could be due to a decrease in phosphodiesterase IV activity, as previously hypothesized [6].

Enoximone, a selective phosphodiesterase IV inhibitor [7], was used to determine whether phosphodiesterase inhibition could differentially enhance β_1-AR- or β_2-AR-mediated responses and whether any such enhancement was altered in tissues from patients on long-term treatment with a β_1-selective antagonist.

Secondary objectives were to determine (a) whether enoximone enhanced responses to catecholamines at concentrations similar to those used in vivo and (b) whether the responses to enoximone were inhibited by the vasodilator diazoxide [8]. This latter question arose because it had been observed in clinical use that concurrent therapy with diazoxide diminished patient responsiveness to enoximone [Latimer and Oduro, pers. commun.].

Methods

The human myocardial tissue was obtained from patients undergoing routine cardiac surgery (coronary artery bypass grafts, valve surgery or both) at Papworth Hospital, UK. Patients receiving β_1-selective antagonists took either atenolol or metoprolol for more than 2 months, up to and including the day of operation.

The method of preparation of atrial strips was as previously described [9]. The right atrial appendage was excised immediately prior to institution of the cardiopulmonary bypass. Tissues were immediately placed in an oxygenated modified Krebs' solution of the following composition (mmol/l): Na^+, 125; K^+, 5; Ca^{2+}, 2.25; Mg^{2+}, 0.5; Cl^-, 98.5; SO_4^{2-}, 0.5; HCO_3^-, 32; HPO_4^{2-}, 1; EDTA, 0.04. Dissection and setting up of the tissues was started within 45 min of surgical removal. Strips with a thickness of less than 1 mm were prepared to facilitate diffusion of oxygen and drugs. Each atrial appendage yielded 2–6 strips. The tissues were mounted in a 50-ml organ bath, at 37 °C, containing a modified Krebs' solution as above, constantly bubbled with 95% O_2 and 5% CO_2 and supplemented with the following (mmol/l): Na^+, 15; fumarate, 5; pyruvate, 5; *L*-glutamate, 5; glucose, 10.

Atrial muscle strips were attached to strain gauge transducers and driven at 2-second intervals with square-wave pulses of 5 ms duration and of just over threshold voltage. A length-force curve was determined and the length set at 40% of the resting tension associated with maximum developed force. Tension developed was recorded on a chart recorder.

The tissues were incubated for 2 h with phenoxybenzamine, 5μmol/l, in order to inhibit uptake of catecholamines and to block myocardial α-AR [9].

Responses to Enoximone

With the tissues in the basal state, cumulative concentration-effect curves were determined for enoximone.