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A comparative study of the cardiovascular and biochemical actions of the imidazo [4,5b] pyridine sulmazole and an imidazo [4,5c] pyridine analogue, BW A746C

G. Allan, [1]D. Cambridge, M.J. Follenfant, *D. Stone & M.V. Whiting

Departments of Pharmacology & *Biochemistry, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS

1 BW A746C is a chemical analogue of the imidazo [4,5b] pyridine, sulmazole (AR-L115 BS). Like sulmazole, BW A746C possesses positive inotropic and vasodilator activity *in vivo*.

2 In anaesthetized guinea-pigs, dogs and primates, a bolus i.v. injection of BW A746C, (0.001–1.0 mg kg^{-1}) caused a significant, dose-related increase in ventricular *dP/dt*, and reduction in diastolic blood pressure, with small increases in heart rate. In these species, a significantly higher dose of BW A746C was required to lower blood pressure by 30% from basal, than was needed to raise ventricular *dP/dt* by 50% over basal.

3 In anaesthetized guinea-pigs and dogs, bolus i.v. injections of sulmazole (0.1–10.0 mg kg^{-1}) caused similar effects to those observed with BW A746C. In these species, however, there was no significant difference between the dose of sulmazole required to lower blood pressure by 30% from basal and that required to raise ventricular *dP/dt* by 50%.

4 In conscious dogs, i.v. infusion of BW A746C (to a total dose of 0.3 mg kg^{-1}) caused a significant increase in ventricular *dP/dt*, but no significant change in either diastolic blood pressure or heart rate.

5 In cell-free biochemical assays, there were no clear differences between the observed activities of BW A746C and sulmazole. Both compounds are cyclic nucleotide phosphodiesterase inhibitors with similar potencies and selectivities for the Type III enzyme (IC_{50} BW A746C = $3.0 \pm 0.5 \times 10^{-5}$ M, sulmazole $5.0 \pm 1.9 \times 10^{-5}$ M). The compounds had little or no effects on sarcolemmal Na^+/K^+-ATPase, Ca^{2+} ATPase or Na^+/Ca^{2+} exchange, and sulmazole, but not BW A746C, had a small, stimulatory effect on myofibrillar ATPase.

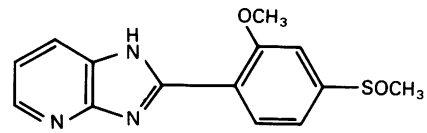
6 In anaesthetized guinea-pigs and dogs, BW A746C was significantly more potent as a positive inotrope than sulmazole. In contrast with sulmazole, BW A746C produced its inotropic effects at significantly lower doses than those required to reduce diastolic blood pressure. This was also apparent from the results obtained in the anaesthetised primates and the conscious dogs. It was therefore concluded that the inotropic/vasodilator profile of BW A746C favours its positive inotrope activity. This profile cannot be explained on the basis of any biochemical differences from sulmazole.

Introduction

The imidazo [4,5b] pyridine, sulmazole (AR-L 115BS), *in vitro* has been found to enhance the contractility of isolated heart muscle (Dahmen & Greef, 1981; Diederen & Weisenberger, 1981; Brutsaert *et al.*, 1982) and to relax isolated vascular smooth muscle (Nguyen Duong *et al.*, 1981). *In vivo* sulmazole has also been shown to increase cardiac contractility in animals (Diederen & Kadatz, 1981; Diederen & Weisenberger, 1981; Dammgen *et al.*, 1981; Pouleur *et al.*, 1983), and in man (Thormann *et al.*, 1982; Renard *et al.*, 1983). These effects *in vivo* are also accompanied by evidence of vasodilator activity.

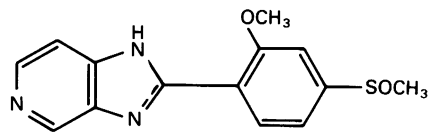
During the study of this compound and chemically related analogues, we observed that this profile of cardiovascular activity could be altered by simple changes in the chemical structure of sulmazole. We have now identified a chemical analogue of sulmazole, an imidazo [4,5c] pyridine (BW A746C; Figure 1) which also has positive inotropic activity *in vitro* (personal communication, R. Hull) and has been

[1] Author for correspondence.



Sulmazole

2-(2-methoxy-4-methylsulphinylphenyl)-1*H*-imidazo [4,5b] pyridine



BW A746C*

2-(2-methoxy-4-methylsulphinylphenyl)-1*H*-imidazo [4,5c] pyridine

Figure 1 The chemical structures of sulmazole and BW A746C. *On completing our studies with BW A746C it was discovered that Eli Lilly and Company had independently developed the same compound which they code-named LY 175326 (see also Discussion).

shown to be a more potent inotrope than sulmazole *in vivo*. In addition we have found evidence to suggest that the vasodilator activity of BW A746C *in vivo* is less pronounced than its inotropic activity. We have therefore examined the cardiovascular effects of BW A746C, in anaesthetized and conscious animals *in vivo*, and compared these with the cardiovascular effects of sulmazole. In addition, we have examined the effects of these two drugs on various biochemical mechanisms which may mediate the myocardial responses to inotropic stimulation.

Methods

Effects of intravenous administration of BW A746C and sulmazole in anaesthetized, closed-chest guinea-pigs

Male guinea-pigs (Hartley-Dunkin) weighing between 450–520 g were anaesthetized by i.p. injection of urethane (BDH, 1.9 g kg^{-1}). The trachea was cannulated and the animal artificially ventilated with room air using a Palmer pump (stroke volume: 1 ml 100 g^{-1} body weight; respiration rate: 40 min^{-1}). A polythene cannula was then inserted into the left jugular vein for subsequent intravenous administration of drugs.

Right ventricular pressure (RVP) and its first derivative, RV*dP/dt*, were measured by passing a polythene cannula (containing heparinised-saline and connected to a Statham pressure transducer) down the right jugular vein into the right ventricular chamber. Arterial blood pressure was measured by placing a second cannula (containing heparinised-saline and connected to a Statham pressure transducer) in the left carotid artery. Heart rate (HR) was derived from this pressure signal by use of a tachograph. Lead II electrocardiogram was obtained using subdermal needle electrodes.

Body temperature was monitored by rectal thermometer and was maintained between 37 and 38°C throughout the experiment by a heated under blanket. All recordings were made by use of a Beckman Type R Dynograph.

In some animals the stability of the preparations was assessed by administering no drugs and monitoring the measured cardiovascular parameters for up to 180 min.

After a 30 min equilibration period, doses of BW A746C (0.01–1 mg kg^{-1}) or sulmazole (0.01–10 mg kg^{-1}), were administered as single bolus i.v. injections in a dose volume of 1 ml kg^{-1}, followed by 0.5 ml of saline. The doses of drugs were given in ascending order and recovery from the effects allowed before administering the next dose. In all animals the response to a bolus injection of vehicle only, was observed.

Effects of intravenous administration of BW A746C and sulmazole in anaesthetized open-chest dogs

Beagle dogs (of either sex) (Cambell Farm, Interfauna Group) weighing between 8.5–13 kg were initially

anaesthetized by an i.v. injection of thiopentone sodium (M & B, 30 mg kg^{-1}) into a cephalic vein. Anaesthesia was subsequently maintained by intravenous injection of α-chloralose (Koch-Light, 15 mg kg^{-1}) and pentobarbitone sodium (Sagatal, M & B, 6 mg kg^{-1}) via a cannula placed in the right femoral vein. The trachea was then cannulated and the animal artificially ventilated with room air using a Palmer pump (stroke volume 200–250 ml and respiration rate 20 min^{-1}). Arterial blood samples were removed before beginning the experiment and analysed (Radiometer Blood Gas Analyser) to ensure that the pump ventilation maintained blood gases within acceptable limits (Green, 1979).

The chest was opened along the length of the sternum and the pericardium opened to expose the heart. The root of the ascending aorta was located and cleared of fat and an electromagnetic flow probe (10–12 mm internal diameter) placed around the ascending aorta and connected to a Statham flowmeter to measure aortic blood flow.

Left ventricular pressure (LVP) and its first derivative, LV$dP/dt$, were measured by the insertion of a short cannula (containing heparinised-saline and connected to a Statham pressure transducer) into the left ventricular chamber via the apex of the heart. This cannula was secured in place by a purse-string suture.

Arterial blood pressure was measured by means of a catheter (containing heparinised-saline and connected to a Statham pressure transducer) inserted into the right femoral artery and a Lead II electrocardiogram was obtained by use of subdermal needle electrodes. HR was derived by use of a tachograph triggered either by the arterial pulse or the ECG-QRS complex. Body temperature was maintained at 37–38°C by a heated under blanket. All recordings were made by use of a Grass Model 7D Polygraph, or a Gould 2800S Recorder.

In some animals the stability of this preparation was assessed by administering no drugs, and monitoring the measured cardiovascular parameters for up to 300 min.

After an initial equilibration period of approximately 60 min, a single bolus i.v. injection of isoprenaline (0.1 μg kg^{-1}) was administered to demonstrate the inotropic reactivity of the preparations. Subsequently a dose-response curve to either BW A746C (0.03–1 mg kg^{-1}) or sulmazole (0.1–3 mg kg^{-1}) was established by administering single bolus i.v. injections in a dose volume of 0.1 ml kg^{-1} in ascending order of doses. Time was allowed for recovery from the effects of each dose before subsequent injections. In some additional animals, the time courses of the effects of a single high dose of either BW A746C (3 mg kg^{-1}) or sulmazole (10 mg kg^{-1}) were also examined. In all animals the response to a bolus injection of vehicle only was observed.

Effects of intravenous administration of BW A746C in anaesthetized closed-chest primates

Female cynomolgus monkeys (Wellcome Research Laboratories) weighing between 3.6–3.8 kg were initially tranquillised with phencyclidine (Sernylan, 2 mg kg^{-1} i.m.) and then anaesthetized by an injection of pentobarbitone sodium (10–20 mg kg^{-1} i.v.) via the cephalic vein. In these studies all animals were allowed to breathe spontaneously.

A polythene cannula (containing saline) was placed in the left femoral vein to allow administration of additional anaesthetic and drugs. A further polythene cannula (containing heparinised-saline, and connected to a Statham pressure transducer) was inserted into the left ventricular chamber, via the left carotid artery, to measure LVP and LV$dP/dt$. A third polythene cannula (also containing heparinised-saline and connected to a Statham pressure transducer) was placed in the left femoral artery to measure arterial blood pressure.

A Lead II electrocardiogram was obtained by use of subdermal needle electrodes and HR was derived from the ECG-QRS complex by use of a tachograph. Body temperature was maintained at 37–38°C by a heated under-blanket. All recordings were made on a Grass model 7D or Beckman Type R Dynograph.

After an initial equilibration period (30 to 60 min after the completion of surgery), a bolus injection of vehicle alone was administered (to ensure that there was no response to injection alone) and a dose-response curve to BW A746C (0.001–1 mg kg^{-1} i.v.) was then established by administering single bolus injections in ascending order of doses.

Because of the limited availability of monkeys no studies were performed to assess the stability of these preparations.

Effects of an intravenous infusion of BW A746C in conscious dogs

Healthy beagle dogs (of either sex) weighing between 8–10 kg were chronically instrumented for measurement of LVP and aortic blood pressure. Animals were initially tranquillised with acepromazine (Berk Pharmaceuticals, 0.15 to 0.5 mg kg^{-1} i.m.), prior to induction of anaesthesia with thiopentone sodium (18 to 25 mg kg^{-1} i.v.) and anaesthesia was maintained with pentobarbitone sodium (10 to 20 mg kg^{-1} i.v.). The animals were then intubated and pump-ventilated with room air.

Using aseptic techniques, a left thoracotomy was performed via the 5th and 6th intercostal space and the heart exposed. A polythene cannula (1 dog) or Konigsberg miniature pressure transducer (4 dogs) was implanted into the left ventricle by means of an apical stab and purse string suture for measurement of LVP.

A polythene cannula introduced into the right or left carotid artery was advanced to the level of the aortic arch or into the descending aorta for measurement of aortic pressure. Cannulae and transducer leads were tunnelled subcutaneously and exteriorised at the back of the neck. The cannulae were filled with heparin-saline and sealed, so as to maintain patency. Wounds were closed with appropriate sutures and covered, and the animals allowed to recover from the anaesthetic. Pethidine (Arnolds, 10 mg kg^{-1} s.c. or i.v.) and diazepam (Roche, 0.5 to 1 mg kg^{-1} i.m.) were given at intervals during the 8 h period following surgery to ease any pain and to restrict movement. All dogs also received a mixture of procaine, penicillin and streptomycin (Streptopen, Glaxo, 500 mg i.m. each).

After recovery periods of at least 6 days, during which time routine checks on body weight, temperature, behaviour, plasma chemistry and haematology were made, the dogs were trained to sit quietly in restraint slings. After laboratory acclimatisation, aortic blood pressure, LVP and LV$dP/dt$ were recorded continuously for periods of up to 8 h. ECG Lead II was obtained by use of subdermal needle electrodes. HR was measured at appropriate intervals, by counting R waves over 0.5 min periods. The recording chart speed was increased periodically for the detection of changes in the ECG wave-form and measurement of the ECG segment intervals (PR, QRS and QT).

On treatment days, an intravenous cannula was inserted percutaneously into either the saphenous or cephalic vein for infusion of either BW A746C, at 3 $\mu g\ kg^{-1}\ min^{-1}$, or the vehicle only (0.2 ml $kg^{-1}\ min^{-1}$) for a period of 2 h. Recordings of aortic blood pressure, LVP, LV$dP/dt$ and ECG-lead II were made for 1 h before and throughout the infusion, then for a further 1 to 2 h afterwards.

Effect of BW A746C and sulmazole on biochemical mechanisms which mediate myocardial responses to inotropic stimulation

The cyclic nucleotide phosphodiesterase activities from dog ventricle were fractionated by DEAE-cellulose chromatography as described by Thompson *et al.* (1979). Inhibitor studies were carried out with both high K_m (Type 1) and low K_m (Type III) enzymes using the assay procedure of Boudreau & Drummond (1975): a substrate concentration of 0.5 μM was employed.

Myocardial sarcolemmal Ca^{2+}-ATPase and Na^+/Ca^{2+} exchange activities were both assayed using an isolated sarcolemmal membrane vesicle preparation from dog ventricles (Van Alstyne *et al.*, 1980). This preparation, which is a mixture of inside-out and right-side out vesicles, has Na^+, K^+-ATPase activities of 22 and 95 μmolPi $mg^{-1}\ h^{-1}$ before and after treatment with sodium dodecyl sulphate, respectively. The final preparation was frozen in liquid N_2 and stored at − 80°C in 10 mM Tris-HCl, pH7.4.

For ATP-dependent uptake, vesicles were resuspended in 160 mM KCl, 20 mM HEPES, pH 7.4. Ca^{2+} uptake was determined at 37°C in a medium containing 160 mM KCl, 5 mM $MgCl_2$, 20 mM HEPES, 10 μM digitoxigenin, 1 $\mu g\ ml^{-1}$ oligomycin, 1 mM ATP, 75 μM $^{45}CaCl_2$, pH 7.4, as described by Caroni & Carafoli (1980). Samples were removed from incubations at 30 s intervals, filtered through Millipore filters (0.22 μm) and washed with 2 × 1 ml of cold incubation medium containing 1 mM $LaCl_3$. Initial rates of Ca^{2+} uptake were measured over 3 min.

For Na^+/Ca^{2+} exchange assays, vesicles were preloaded with 140 mM NaCl, 10 mM HEPES, pH 7.4. Exchange assays were carried out essentially as described by Philipson (1984). Assays were initiated by rapid dilution of vesicles into a medium containing 140 mM KCl, 10 mM HEPES, 20 μM $^{45}CaCl_2$, pH;7.4 and terminated by the addition of 1 mM $LaCl_3$ (final concentration): a metronome set to beat at 1 s^{-1} was used to time these incubations. Samples were filtered (0.22 μm, Millipore) and washed with 2 × 3 ml of 140 mM KCl, 0.1 mM $LaCl_3$. The time course of exchange over 4 s was linear and was used to estimate the initial rate.

The preparation of Na^+, K^+-ATPase from dog ventricles was carried out as described by Pitts & Schwartz (1975). Enzyme activity was assayed spectrophotometrically by use of a pyruvate kinase/lactic dehydrogenase coupled assay (Schwartz *et al.*, 1969).

Sarcoplasmic reticulum was prepared from minced dog ventricle as described by Schwartz *et al.* (1984). The ouabain-sensitive Na^+, K^+-ATPase of this preparation is approximately 5 μmol Pi $mg^{-1}\ h^{-1}$ indicating only a low level of contamination with sarcolemmal membrane. ATP-dependent uptake of Ca^{2+} was determined at 37°C in a medium containing 0.1 M KCl, 4 mM $MgCl_2$, 30 μM $^{45}CaCl_2$ 3.5 mM ATP, 5 mM potassium oxalate, 5 mM NaN_3, 0.1 M sucrose, 20 mM imidazole-HCl, pH 6.8, by filtration through 0.22 μm Millipore filters as described by Chamberlain *et al.* (1983). Rates of accumulation of ^{45}Ca were measured over 5 min.

Myofibrils were prepared from guinea-pig ventricles by the procedure of Solaro *et al.* (1971). Ca^{2+}-dependent Mg^{2+}-ATPase was determined from the rate of production of Pi over 10 min at 30°C in a medium containing 50 mM KCl, 2 mM ATP, 2 mM $MgCl_2$, 1 mM EGTA, 20 mM imidazole-HCl, pH 7.0 and various $CaCl_2$ concentrations calculated to give a range of concentrations of free Ca^{2+} between 10^{-8} and 10^{-5} M. Stability constants for Ca/EGTA were calculated as described by Fabiato & Fabiato (1979).

Drugs used

BW A746C and sulmazole were synthesized by Drs W.R. King and R. Iyer in the Department of Medicinal Chemistry, Wellcome Research Laboratories, Beckenham. These drugs were administered in aqueous solution (either distilled water or 0.9% saline to which was added a small quantity of 0.1 N HCl to aid solubility).

Analysis of data

The cardiovascular responses to each dose of BW A746C or sulmazole in anaesthetized animals were measured as the percentage change from predose levels. A mean dose-response curve was then constructed so that each point on this curve was the mean (± s.e.mean) for a group of animals. In these studies it was not possible to define the maximum responses to these agents, therefore, using the dose-response data derived from the individual animals, the dose of BW A746C or sulmazole to cause an increase in ventricular dP/dt of 50% above basal (DI_{50}) and a decrease in diastolic blood pressure of 30% below basal (DP_{30}), was calculated. The mean (± s.e.mean) DI_{50} and DP_{30} for a group of animals could then be calculated from these individual values.

Where statistical analyses of these data have been carried out, these have utilised a local software package (compiled by the Computing and Statistical Services Department of the Wellcome Research Laboratories) and Student's t test applied (paired for intra-group analyses unpaired for inter-group analyses). Statistical significance was assumed at P values <0.05.

In the biochemical studies the effects of drugs on the steady state rates of enzyme or ion transport processes were determined: all assays were linear with respect to time in the presence and absence of drug. Where I_{50} values could be determined they were calculated by least square fitting of the data to the model

$$Vi = Vo/[1 + (I/I_{50})^n] + C$$

where Vo and Vi are enzyme rates in the absence of drug and in the presence of drug at concentration I respectively, I_{50} is the concentration of drug required to give 50% inhibition of activity under the chosen assay conditions, and n and C are constants. Values are quoted as the mean ± s.e.mean.

Results

Effects of intravenous administration of BW A746C and sulmazole in anaesthetized closed-chest guinea-pigs

In animals which received no drug, there was a gradual increase from zero time in resting RV$dP/dt$ and diastolic blood pressure (DBP) over the experimental period of 180 min but these only reached statistical significance at times beyond 150 min. However, there was very little change in HR. Therefore no corrections for temporal changes in baselines were made when analysing the cardiovascular responses to bolus injections of BW A746C or sulmazole obtained in subsequent experiments.

In these animals, the mean basal values for the measured cardiovascular parameters were 608 ± 31 mmHg s^{-1} (RV$dP/dt$), 24 ± 1 mmHg (DBP) and 293 ± 10 beats min^{-1} (HR). Both BW A746C (0.1–1 mg kg^{-1}) and sulmazole (0.01–10 mg kg^{-1}) produced a dose-related increase in RV$dP/dt$, and reduction in DBP, associated with some increase in HR. These effects are summarized in Figure 2. Neither agent caused any gross changes in the ECG.

No consistent definitions of the maximum responses to these agents were obtained in these studies therefore a mean DI_{50} and DP_{30} were calculated as described above (see Analysis of Data and Table 1). The DI_{50} for BW A746C was significantly lower than its DP_{30} suggesting that the inotropic activity of this agent occurred at lower doses than did its vasodilator activity. By contrast, there was no significant difference between the DI_{50} and DP_{30} for sulmazole. The DI_{50} and DP_{30} for BW A746C were also lower than those for sulmazole; however, this was only significant for the DI_{50}.

Effects of intravenous administration of BW A746C and sulmazole in anaesthetized open-chest dogs

In animals which received no drug there was little change in the measured cardiovascular parameters over the experimental period of 300 min. There was a gradual increase in HR which eventually attained statistical significance, but no corrections for temporal changes in basal levels were made when analysing the cardiovascular responses to individual doses of BW A746C or sulmazole in subsequent experiments.

In these animals, the mean basal values for the measured cardiovascular parameters were 2913 ± 178 mmHg s^{-1} (LV$dP/dt$), 5795 ± 637 ml min^{-1} (peak aortic blood flow), 93 ± 4 mmHg (DBP) and 185 ± 3 beats min^{-1} (HR). Both BW A746C (0.03–1 mg kg^{-1}) and sulmazole (0.1–3 mg kg^{-1}) produced dose-related increases in LV$dP/dt$ and peak aortic blood flow, and reduced DBP in association with some increase in HR. These effects (except those of peak aortic blood flow, which are very similar to those of LV$dP/dt$) are summarized in Figure 3. Neither agent caused any gross changes in the ECG.

No consistent definitions of the maximal responses to these agents were obtained in these studies, therefore mean DI_{50} and DP_{30} values were calculated as

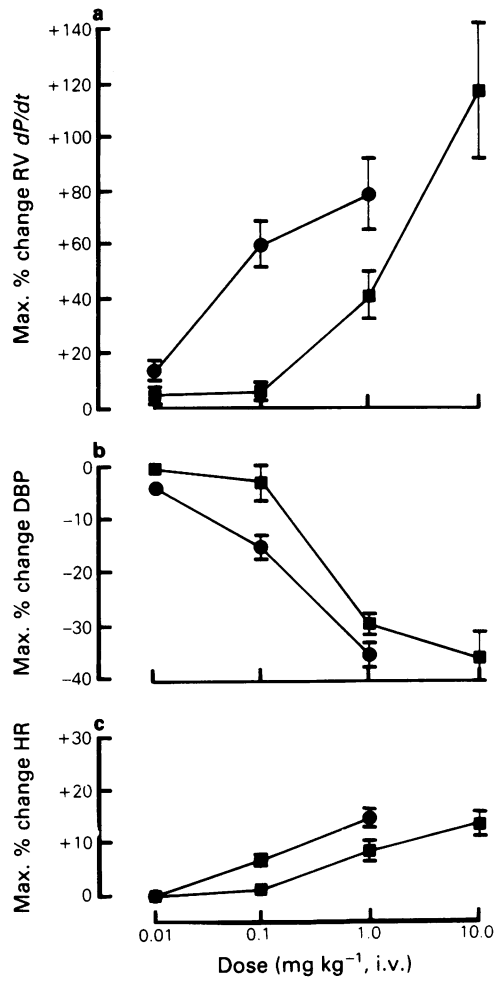


Figure 2 The effects of intravenous administration of BW A746C (●) and sulmazole (■) in anaesthetized, closed-chest guinea-pigs on (a) right ventricular (RV) *dP/dt* (b) diastolic blood pressure (DBP) and (c) heart rate (HR). Each point is the mean for 6 animals and vertical lines indicate s.e.mean.

Table 1 Mean dose of BW-A746 C and sulmazole to produce a 50% increase in ventricular *dP/dt* (DI_{50}) over basal and a 30% decrease in diastolic blood pressure (DP_{30}) below basal

	DI_{50} (mg kg^{-1})	DP_{30} (mg kg^{-1})
Guinea-pigs		
	$P<0.05$	
BW A746C ($n=6$)	0.094 ± 0.032	0.68 ± 0.15
	$P<0.05$	NS
Sulmazole ($n=6$)	1.61 ± 0.46	1.15 ± 0.21
	NS	
Dogs		
	$P<0.05$	
BW A746C ($n=9$)	0.067 ± 0.011	0.25 ± 0.07
	$P<0.05$	$P<0.05$
Sulmazole ($n=4$)	1.31 ± 0.56	1.46 ± 0.52
	NS	
Primates		
	$P<0.05$	
BW A746C ($n=3$)	0.061 ± 0.04	>1.0

Values for DI_{50} and DP_{30} are expressed as mean ± s.e.mean. Statistical analysis of these data utilised the paired and unpaired Student's *t* tests (see Analysis of Data). $P<0.05$ indicates statistical significance, NS indicates no statistical significance.

described above (see Analysis of Data and Table 1). In these studies, the DI_{50} for BW A746C was significantly lower than its DP_{30}, indicating that the inotropic activity of this agent occurred at lower doses than did its vasodilator activity. By contrast, there was no significant difference between the DI_{50} and DP_{30} for sulmazole. The DI_{50} and DP_{30} for BW A746C were also significantly lower than their counterparts for sulmazole.

In some additional animals, the time courses of the responses to a single large bolus dose of BW A746C (3 mg kg^{-1}) or sulmazole (10 mg kg^{-1}) were observed. The maximal responses to these agents were achieved within 5 min of administration. BW A746C ($n=3$) and sulmazole ($n=3$) produced similar increases in LV*dP/dt* (112 ± 38% vs 110 ± 26%, respectively) and reductions in DBP (55 ± 3% vs 52 ± 4%, respectively). These effects were also accompanied by increases in peak aortic blood flow (83 ± 10% vs 55 ± 13%, respectively) and HR (32 ± 4% vs

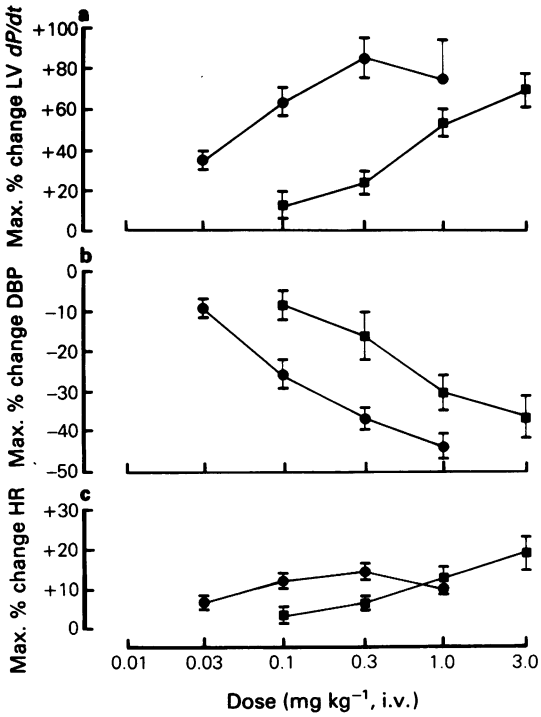


Figure 3 The effects of intravenous administration of BW A746C (●) and sulmazole (■) in anaesthetized, open-chest dogs on (a) left ventricular (LV) *dP/dt*, (b) diastolic blood pressure (DBP) and (c) heart rate (HR). Each point is the mean for 9 (BW A746C) or 4 (sulmazole) animals and vertical lines indicate s.e.mean.

41 ± 7%, respectively). Despite an initial decline in these responses, the inotropic effects of BW A746C were still evident 180 min after administration (66 ± 13% increase in LV*dP/dt* over basal), as were the vasodilator effects (46 ± 7% decrease in DBP below basal). By contrast, the inotropic and vasodilator effects of sulmazole had almost completely disappeared after this period of time.

Effects of intravenous administration of BW A746C in anaesthetized closed-chest primates

In these animals, the mean basal levels for the measured cardiovascular parameters were 5093 ± 173 mmHg s^{-1} (LV*dP/dt*), 84 ± 2 mmHg (DBP) and 210 ± 2 beats min^{-1} (HR). BW A746C (0.001–1 mg kg^{-1}) produced a dose-related increase in LV*dP/dt* which appeared to reach a maximum at a dose of 0.3 mg kg^{-1}. This inotropic effect was accompanied by a dose-related, but less pronounced, reduction in DBP and an increase in HR. The effects of BW A746C are summarized in Figure 4. In these animals, BW A746C caused no gross changes in the ECG.

Although evidence for a maximal response to BW A746C was obtained in these animals, in order to maintain consistency with the results obtained in guinea-pigs and dogs, a mean DI_{50} and DP_{30} were calculated, as described previously (see Analysis of Data and Table 1). In these studies, the DI_{50} for BW A746C, was significantly lower than the DP_{30}, indicating that the inotropic activity of this agent occurred at lower doses than did its vasodilator activity.

Effects of an intravenous infusion of BW A746C in conscious dogs

In these animals the mean basal levels for the measured cardiovascular parameters were 4398 ± 468 mmHg s^{-1} (LV*dP/dt*), 75 ± 5 mmHg (DBP) and 84 ± 2 beats min^{-1} (HR). BW A746C (3 μg kg^{-1} min^{-1}) produced an increase in LV*dP/dt* within 10 min of beginning the infusion. LV*dP/dt*

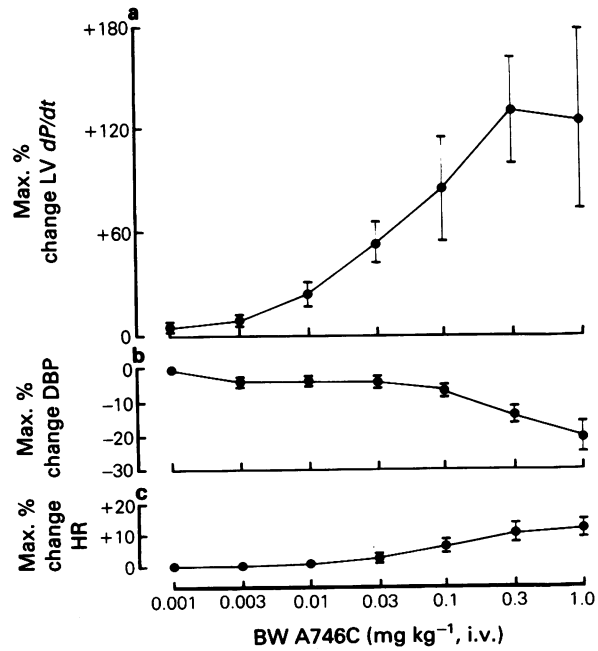


Figure 4 The effects of intravenous administration of BW A746C in anaesthetized, closed-chest primates on (a) left ventricular (LV) *dP/dt*, (b) diastolic blood pressure (DBP) and (c) heart rate (HR). Each point is the mean for 3 animals and vertical lines indicate s.e.mean.

continued to increase thereafter but eventually reached a maximum (63 ± 8%, $P<0.05$ by Student's paired t test) approximately 100 min after beginning the infusion. On cessation of the infusion, LV$dP/dt$ gradually returned to basal levels. Despite this significant cardiac effect, BW A746C caused no change in either DBP or HR. These cardiovascular effects are summarised in Figure 5 and support the premise that the cardiovascular effects of BW A746C favour its positive inotropic activity.

In these studies BW A746C produced no gross changes in the ECG, but it was observed that its cardiovascular effects were also associated with a significant decrease in the atrio-ventricular conduction time (reduction of 16 ± 3% in PR-interval).

Effect of BW A746C and sulmazole on biochemical mechanisms which mediate myocardial responses to inotropic stimulation

The effects of BW A746C and sulmazole on a number of cell-free biochemical systems, which might be relevant to the myocardial response to these drugs are summarized in Table 2.

BW A746C and sulmazole had no significant effects on the initial rate (over 4 s) or the maximal capacity (after 2 min) of Na^+/Ca^{2+} exchange in Na^+-loaded sarcolemma vesicles at concentrations up to 10^{-4} M. In the case of BW A746C, some inhibition of the rate of exchange was observed at higher concentrations of drug. A very similar pattern was observed for inhibition of ATP-dependent Ca^{2+} uptake by K^+-loaded sarcolemmal vesicles. In both assays essentially identical results were obtained with or without pretreatment of vesicles for 30 min with drug. The compounds were also ineffective as inhibitors of sarcolemmal Na^+, K^+-ATPase and of ATP-dependent Ca^{2+} uptake by sarcoplasmic reticulum, though BW A746C showed some inhibition of both activities at 10^{-3} M.

The effects of both drugs on the Ca^{2+}-stimulated Mg^{2+}-ATPase of isolated myofibrils were determined using a range of free Ca^{2+} concentrations between 10^{-8} and 10^{-5} M. In the absence of drug the ATPase activity was half maximal at 1 μM free Ca^{2+}. BW A746C at concentrations up to 10^{-4} M had no effect on the ATPase activity at any concentration of Ca^{2+} tested. As shown in Table 2, however, 10 μM sulmazole gave a small but consistent stimulation of ATPase activity at 1 μM Ca^{2+}, although at Ca^{2+} concentrations above 5 μM, the drug had no effect.

Both compounds were effective inhibitors of both Type I and Type III adenosine 3':5'-cyclic monophosphate (cyclic-AMP) phosphodiesterases from dog ventricle. The potency of both compounds was similar and they both showed some selectivity for the Type III enzymes.

Discussion

In the anaesthetized and conscious animal studies presented in this paper, we have confirmed the reported inotropic and vasodilator properties of the imidazo [4,5b] pyridine, sulmazole, and found that an analogue, the imidazo [4,5c] pyridine BW A746C, also possesses these properties. However, where the cardiovascular effects of these agents have been compared, it has been observed that BW A746C is significantly more potent as a positive inotrope than sulmazole (in excess of 10 fold). In addition, the dose of BW A746C, but not sulmazole, to produce a standard inotropic response (50% increase in ventricular dP/dt) is significantly less than that required to produce a standard vasodilator response (30% decrease in diastolic blood pressure). These results therefore indicate that the cardiovascular profile of BW A746C favours its inotropic properties, whereas for sulmazole the inotropic and vasodilator properties occur at similar doses.

During the course of our studies with BW A746C, it was learnt that the cardiovascular properties of this agent had been subjected to an independent investigation. A subsequent study has confirmed our findings with this agent (code named LY 175326 in this study – Hayes *et al.*, 1985a). In anaesthetised cats and dogs, it

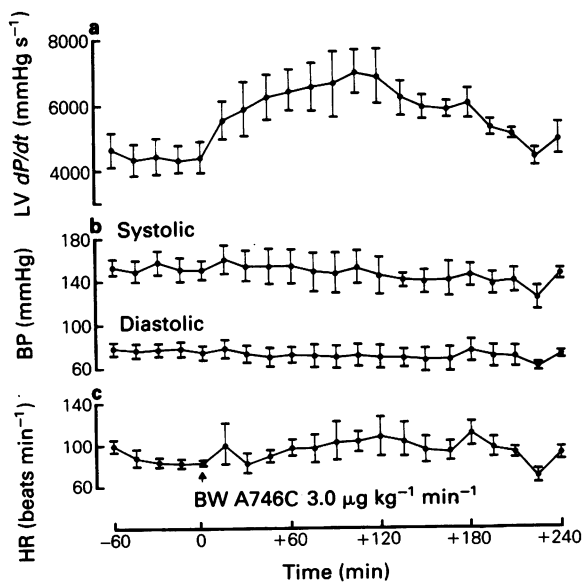


Figure 5 The effects of an intravenous infusion of BW A746C in conscious dogs on (a) Left ventricular (LV) dP/dt, (b) blood pressure (BP) and (c) heart rate (HR). Each point is the mean for 4–5 animals and vertical lines indicate s.e.mean.

was a more potent inotrope than sulmazole (in excess of 10 fold) and produced its inotropic effects at significantly lower doses than its vasodilator effects. Thus a minor chemical modification in the heterocyclic imidazo-pyridine base of sulmazole increases the inotropic potency and may also modify the cardiovascular profile *in vivo*.

Despite the close similarity in the chemical structure of sulmazole and BW A746C (see Figure 1), there are significant differences in their physicochemical and molecular properties. The modification of the heterocyclic base from sulmazole to produce BW A746C does not alter the lipophilicity of the molecule but it does markedly increase the basicity (pK_A sulmazole: BW A746C = 3.9:6.2) and alters the sites of protonation and the predominant tautomeric species (personal communication – P. Barraclough). Therefore, although the importance of these physicochemical and molecular properties in determining the cardiovascular effects of these imidazo-pyridines is not fully understood, from our results it appears that changes in these properties are associated with changes in inotropic potency and cardiovascular profile *in vivo*.

The mechanism by which the imidazo-pyridines produce their inotropic effects is not yet determined. Most studies with sulmazole have indicated that a glycoside-like mechanism can be ruled out (Diederen & Weisenberger, 1981; From & Pierpoint, 1981; Hayes *et al.*, 1985b), although Weishaar *et al.* (1983) have demonstrated inhibition of myocardial Na^+-K^+ ATPase by sulmazole at concentrations which are inotropic *in vitro*. In the studies presented here, inhibition of this APTase by sulmazole and BW A746C was also observed, but only at high doses. It therefore seems unlikely that these agents act primarily through this mechanism.

The involvement of cardiac β-adrenoceptors is less equivocal. Although Brutsaert *et al.* (1982) have demonstrated that the inotropic effects of sulmazole are attenuated by β-adrenoceptor blockade *in vitro*, this requires high doses of the β-adrenoceptor blocking agents used. The majority of studies have found no interaction between sulmazole and β-adrenoceptor blockers (Diederen & Weisenberger, 1981; From & Pierpoint, 1981; Hayes *et al.*, 1985b). In our studies there was also no evidence for a β-adrenoceptor-mediated inhibition of the inotropic effects of sulmazole or BW A746C (unpublished observations). The location of the inotropic dose-response curve to both agents was unchanged by the presence of β-adrenoceptor blocking agents in guinea-pig ventricle *in vitro* and in anaesthetized dogs *in vivo*. This has also been demonstrated for BW A746C by Hayes *et al.* (1986). Thus it seems that these agents do not act through the stimulation of myocardial β-adrenoceptors.

The movement of Ca^{2+} into, and within, the myocardial cell plays a fundamental role in the development of contractile tension. This Ca^{2+} movement is influenced directly by inotropic agents which act through glycoside, or β-adrenoceptor mechanisms. Evidently this is not the case for the imidazo-pyridines, as these agents do not appear to act through either of these mechanisms. Nevertheless, it is possible that they influence this Ca^{2+} movement

Table 2 Comparison of the activities of BW A746C and sulmazole on some isolated cell-free systems which may mediate responses to inotropic stimulation

Activity	*Inhibition (%) of control values at maximum concentration of drug tested or IC_{50}**	
	BW A746C	*Sulmazole*
Cyclic AMP phosphodiesterase (Type 1)	$6.2 \pm 2.0 \times 10^{-4}$ M*	$2.9 \pm 1.2 \times 10^{-4}$ M*
Cyclic AMP phosphodiesterase (Type III)	$3.0 \pm 0.5 \times 10^{-5}$ M*	$5.0 \pm 1.9 \times 10^{-5}$ M*
Sarcolemma Ca^{2+} ATPase	45 ± 7 at 10^{-3} M	No effect at 10^{-4} M
Sarcolemma Na^+/Ca^{2+} exchange	40 ± 8 at 10^{-3} M	No effect at 10^{-4} M
Na^+, K^+-ATPase	30 ± 10 at 10^{-3} M	17 ± 5 at 10^{-4} M
Sarcoplasmic reticulum Ca^{2+} uptake	20 ± 4 at 10^{-3} M	NT
Myofibrillar ATPase (1 μM Ca^{2+})	No effect at 10^{-4} M	16 ± 7 stimulation at 10^{-4} M

*Concentration required for 50% inhibition of activity (IC_{50}) under the assay conditions described in Methods. NT: not tested.

directly by acting upon myocardial cellular structures such as the sarcolemma or the sarcoplasmic reticulum. Weishaar *et al.* (1983) and Hayes *et al.* (1986) have been unable to find any convincing evidence for such an action for either sulmazole or BW A746C. Our own studies have found only weak actions for sulmazole and BW A746C upon these structures (Na^+/Ca^{2+}-exchange and Ca^{2+}-ATPase activity at the sarcolemma; Ca^{2+}-uptake at sarcoplasmic reticulum). Thus it appears unlikely that the imidazo-pyridines exert their positive inotropic effects through a direct action upon either the sarcolemma or sarcoplasmic reticulum. However the Ca^{2+} movement that is controlled by these structures may be influenced indirectly through the elevation of intracellular cyclic AMP. This can be achieved by the stimulation of myocardial adenyl cyclase (as with β-adrenoceptor stimulation) or by inhibition of myocardial cyclic AMP-phosphodiesterase. There is little evidence to support the notion that the imidazo-pyridines act by stimulating adenyl cyclase (Hayes *et al.*, 1986), but it has been shown that sulmazole inhibits myocardial cyclic AMP-phosphodiesterase (Diederen & Wiesenberger, 1981; Weishaar *et al.*, 1983). However this enzyme exists as at least three isoenzymes, of which the most important, with regard to positive inotropic stimulation, is that described as PDE-III. Several potent inotropes, including milrinone, MDL-17043 and Cl-914, have been identified which show a marked selectivity for this isoenzyme (Kariya *et al.*, 1982; Bristol *et al.*, 1984; Weishaar *et al.*, 1985). More recently both sulmazole (Bristol *et al.*, 1984) and BW A746C (Hayes *et al.*, 1986) have been found to have some selectivity for the PDE-III isoenzyme. Our studies have also confirmed this selectivity for sulmazole and BW A746C for PDE-III over PDE-I isoenzyme. However, there is very little difference in their potency as inhibitors of PDE-III isoenzyme. In addition, when compared with other more potent inhibitors of this isoenzyme, such as MDL-17043, it is interesting to note that BW A746C is a more potent positive inotrope (unpublished observations). Thus it appears that the inhibition of PDE-III isoenzyme by these imidazo-pyridines is inadequate to explain all of their positive inotropic activity. However, it is becoming clear that this inhibition will at least contribute to an increased availability of intracellular Ca^{2+} within the myocardial cells and must, therefore, also contribute to the inotropic activity of these agents.

There is also some evidence to suggest that the imidazo-pyridines increase the sensitivity of the contractile machinery of myocardial cells to Ca^{2+}. Sulmazole has been demonstrated to increase the sensitivity of the myofibrils to Ca^{2+} (Herzig *et al.*, 1981; Solaro & Ruegg, 1982; Blinks & Endoh, 1984). This would have the same net effect on the development of contractile tension, as would an increase in the availability of intracellular Ca^{2+}. Two structurally dissimilar inotropic agents, APP 201-533 (Salzmann *et al.*, 1985) and DPI 201-106 (Scholtysik *et al.*, 1985) have also been found to exhibit this property. In our own studies, we have observed that sulmazole stimulates myofibrillar ATPase, which is consistent with these other findings. However, no such effect was observed with BW A746C. As BW A746C is a more potent inotrope than sulmazole, these observations would suggest that for BW A746C, at least, this myofibrillar sensitization is not important. It therefore remains to be seen whether this is an important property in sulmazole, which does indeed contribute to its positive inotropic activity.

If the effects of BW A746C and sulmazole are due primarily to myocardial cyclic AMP-phosphodiesterase inhibition, it seems unlikely that the rather small differences in inhibitory potency and selectivity for the PDE-III isoenzyme could account for the different cardiovascular profiles of the two compounds. Such differences may therefore be a function of differences of access and disposition within the cardiovascular system rather than of different properties at the biochemical level.

The clinical application of inotropes results primarily from the clear evidence of an impaired pumping ability of the heart, as the primary derangement in congestive heart failure (Weber & Janicki, 1978). However, the compensatory mechanisms (i.e. vasoconstriction, salt and water retention etc.,) initiated by the cardiovascular system, in response to the progressive failure of the heart, ensure that an increased loading is placed upon an inefficient, 'sick' heart, which can only result in accelerating its deterioration. Consequently, it has been successfully argued that vasodilators (arterial, venous or both) may be more beneficial as they reduce the loading on the 'sick' heart, removing the constraints on an inefficient pump. Thus in the absence of any drugs, other than cardiac glycosides, which could be considered as pure inotropes, the use of vasodilators has become more widely accepted (Mason, 1978; Cohn & Franciosa, 1978). The advent of new, non-glycoside, non-catecholamine, chemical structures which possess positive inotropic activity has revived the hope that the relative merits of these two therapeutic approaches could be more carefully compared. Unfortunately many of these new structures have also been found to possess vasodilator activity (Colucci *et al.*, 1986). Our observations that within the imidazo-pyridines it is possible to establish different cardiovascular profiles, despite an unknown mechanism of action, is encouraging. A direct comparison of the merits of an inotrope, or vasodilator, or mixed inotrope-vasodilator in the treatment of heart failure, may therefore be possible in the near future.

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