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BW A575C, a chemically novel agent with angiotensin converting enzyme inhibitor and β-adrenoceptor-blocking properties

[1]G. Allan, D. Cambridge, G.W. Hardy & M.J. Follenfant

Departments of Pharmacology I and Medicinal Chemistry, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS

1 BW A575C (N-(1-(S)-carboxy-5-[4(3-isopropylamino-2-(R, S)-hydroxypropoxy)indole-2-carboxamido]pentyl)-(R, S)-alanyl-(S)-proline) is a chemically novel agent which exhibits in a single molecule both angiotensin converting enzyme (ACE) inhibition and β-adrenoceptor-blocking properties.

2 BW A575C produced a competitive blockade of heart rate responses to isoprenaline in a guinea-pig right atrial preparation (pK_B 7.18 ± 0.05, cf. pindolol 8.9 ± 0.7). BW A575C inhibited a partially purified preparation of ACE obtained from rabbit lung (IC_{50} 10.7 ± 2.1 nM, cf. enalaprilat, 4.4 ± 0.8 nM).

3 Intravenous administration of BW A575C (1–100 μg kg^{-1} min^{-1}) to the pithed rat inhibited in a dose-dependent fashion both angiotensin I-induced pressor responses and isoprenaline-induced tachycardia. Dose-ratios obtained from such studies demonstrated that, in this preparation, BW A575C was approximately 100 times more active as an ACE inhibitor than as a β-adrenoceptor blocking agent.

4 Intravenous administration of BW A575C (1 mg kg^{-1}) to the conscious rat inhibited angiotensin I-induced pressor responses, being approximately equipotent to enalapril and 10 times more potent than captopril. At the same dose, BW A575C had a similar duration of action as an ACE inhibitor to enalapril.

5 Intravenous administration of BW A575C (1 mg kg^{-1}) to either conscious dogs or rats inhibited both angiotensin I-induced pressor responses and isoprenaline-induced heart rate responses. Dose-ratios obtained from such studies demonstrated that in these species, BW A575C was 2–10 times more active as an ACE inhibitor than as a β-adrenoceptor blocking agent.

Introduction

Both angiotensin converting enzyme (ACE) inhibitors and β-adrenoceptor blocking agents have become established as effective and well tolerated antihypertensive agents (Case *et al.*, 1978, Prichard & Owens, 1986). Although these agents have been shown to reduce blood pressure in several forms of human hypertension, a number of studies have demonstrated that hypertension cannot be completely controlled by either type of drug alone. As a consequence, multiple drug therapy using a variety of therapeutic agents remains as the common approach to the treatment of hypertension (Moser, 1977).

In order to overcome the limitations of the available antihypertensive drug therapy there has emerged a number of agents which represent a new class of drugs referred to as 'hybrid' drugs that combine in one molecule two distinct pharmacological activities (Nicolaus, 1983). The antihypertensive agents, labetalol, which combines α- and β-adrenoceptor blocking properties (Brittain *et al.*, 1982) and prizidilol, which combines vasodilator and β-adrenoceptor-blocking properties (Taylor *et al.*, 1981) are examples of this class of drug.

We would like to describe the results of studies with a chemically novel agent, BW A575C (see Figure 1) which combines in a single molecule ACE inhibitor properties with β-adrenoceptor-blocking properties. Whereas both ACE inhibitors and β-adrenoceptor-blocking agents can independently reduce blood pressure via different mechanisms, their co-administration may be complementary when considering their actions within the renin-angiotensin system (Prichard &

[1]Author for correspondence (Dept. of Pharmacology I).

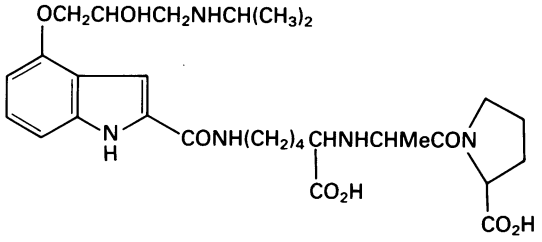

Figure 1 The chemical structure of BW A575C

Owens, 1986; Johnston, 1986). Inhibition of ACE causes a reduction in the circulating levels of the pressor hormone angiotensin II, however, treatment with such agents results in an increase in renin release. Whilst the different factors involved in the regulation of the renin response evoked by ACE inhibitors are unclear, the increase in renin secretion has been demonstrated to be sensitive to β-adrenoceptor blockade (Schiffrin *et al.*, 1981; Pickering *et al.*, 1982; Ferguson *et al.*, 1983; MacGregor *et al.*, 1985). Therefore, an agent such as BW A575C which possesses both ACE inhibitor properties and β-adrenoceptor blocking properties may have an improved therapeutic profile over an ACE inhibitor or β-adrenoceptor blocker alone by controlling more effectively renin secretion. This paper describes the dual pharmacological action of BW A575C both *in vitro* and *in vivo* and compares it with the angiotensin converting enzyme inhibitor, enalapril and the β-adrenoceptor blocking agent, pindolol.

Methods

ACE inhibition and β-adrenoceptor blocking studies in vitro

Guinea-pig atrial preparation The β-adrenoceptor blocking properties of BW A575C were studied in isolated, spontaneously beating right atria from male guinea-pigs (350–425 g). The animals were killed by cervical dislocation and the hearts rapidly excised and washed in Krebs solution of the following composition (mM): Na^+ 143, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128, $H_2PO_4^-$ 2.2, HCO_3^- 24.9, SO_4^{2-} 1.2 and dextrose 10. The atria were dissected free and transferred to a glass dish containing the same solution gassed with 95% O_2 : 5% CO_2, the apex of the right atrium was tied to a stainless steel wire with cotton while a second ligature was tied to the base of the atrium. The tissue was suspended in a 20 ml pyrex organ bath containing the same buffer and maintained at 37°C (± 0.3°C). The lower ligature was held in a perspex clamp and the wire suspended from an isometric transducer (ADG Instruments). Transducer output was processed by a ratemeter (ADG Instruments) which gave a direct readout of rate continuously displayed on a potentiometric recorder (Bryans 18000). Tissues were subjected to 0.5 g resting diastolic tension and washed at approximately 15 min intervals until a steady basal rate (variation of less than 5 beats min^{-1} in a 15 min period) was obtained. Atria showing basal rates of less than 140 beats min^{-1} or greater than 280 beats min^{-1} were discarded. Tissues were incubated for 60 min with BW A575C or pindolol at various concentrations before starting a cumulative isoprenaline concentration-effect curve. Control and treatments were randomised according to a 6 × 6 latin square design.

Rabbit lung ACE activity Angiotensin converting enzyme (ACE) was partially purified from rabbit lung acetone powder prepared as described previously (Reynolds, 1984). The activity of this enzyme was then measured by a spectrophotometric assay based on the hydrolysis of 2-furanacrylolyl-L-phenylalanyl-glycyl-glycine (FA-PGG), as described by Holmquist *et al.* (1979).

Cuvettes (0.5 ml) containing 0.2 mM FA-PGG, 50 mM Tris-HCl/0.3M NaCl buffer at pH 7.5, and inhibitor drug, as desired, were used in these studies. The partially purified enzyme was then added to initiate the hydrolysis reaction. The hydrolysis was monitored continuously by the decrease in absorbance at 355 nm in a thermostatically controlled (30°C) spectrophotometer (Gilford 250).

To evaluate the inhibition of ACE, the velocity for the hydrolysis in the presence and absence of inhibitor drug, was estimated from the recorder trace obtained between 3 and 7 min after initiation. The apparent IC_{50} value (concentration of inhibitor drug to reduce the activity of ACE by 50%) was estimated from experiments where three or more concentrations of inhibitor drug were used. In these studies the IC_{50} for BW A575C, and enalapril (as the diacid enalaprilat) were measured.

ACE inhibition and β-adrenoceptor blocking studies in vivo

Pithed rat studies Male Wistar rats (180–320 g) were anaesthetized with a halothane/air mixture and the trachea cannulated. Animals were pithed and placed on a small surgical table. A carotid artery was cannulated for continuous measurement of blood pressure via a blood pressure transducer (Statham) which was displayed with integrated heart rate on a polygraph trace (Beckman Dynograph). Both jugular veins were cannulated, one to infuse inhibitor drugs and the other for bolus injection of either angiotensin I (0.01–10 μg kg^{-1}) or isoprenaline (0.001–3 μg kg^{-1}).

Each animal received either vehicle or test drug infused at a rate of 0.03 ml min^{-1} via a jugular cannula. After an infusion period of 15 min a dose-response curve to angiotensin I or isoprenaline was started.

Doses of angiotensin I or isoprenaline were administered intravenously in a constant volume of 0.1 ml in an increasing sequence of dose. Only one dose-response curve was obtained from each animal, vehicle or inhibitor drug-treatment being randomized.

Conscious rat studies Conscious male normotensive Wistar rats (280–320 g, $n = 6$) with indwelling femoral arterial and venous cannula (implanted under halothane/air anaesthesia on the day of experiment) were housed in a confined grid structure and placed in perspex boxes to restrict movement. Blood pressure and integrated heart rate were continuously recorded (Beckman Dynograph or Gould 800s) via a blood pressure transducer (Statham) connected to the arterial cannula. Each animal received either vehicle or test drug by bolus intravenous injection 30 min before a dose-response curve to either angiotensin I or isoprenaline was started.

Bolus intravenous injections of angiotensin I (0.003–3 $\mu g\,kg^{-1}$) or isoprenaline (0.003–3 $\mu g\,kg^{-1}$) were administered in a constant volume of 0.1 ml, and in an increasing sequence of dose. Only one dose-response curve was obtained from each animal, vehicle or drug treatment being randomised.

In some experiments, the time course of action of either BW A575C or enalapril was determined by measuring the recovery of a sub-maximal pressor response, evoked by acute intravenous administration of angiotensin I (0.3 $\mu g\,kg^{-1}$), after pretreatment of animals with equieffective doses of either BW A575C or enalapril (1 mg kg^{-1}).

Conscious dog studies Conscious normotensive beagle dogs of either sex (8–12 kg, $n = 5$) with an indwelling aortic cannula (implanted under general anaesthesia at least 10 days before the experiment) and a cephalic or saphenous venous cannula (implanted by acute percutaneous puncture) were placed in specially designed laboratory restraint slings. Blood pressure and integrated heart rate were continuously recorded (Gould 2800s) via a blood pressure transducer (Statham) connected to the aortic cannula. Each animal received BW A575C by bolus intravenous injection after obtaining within the same animal a control dose-response curve to angiotensin I and isoprenaline. A repeat dose-response curve to angiotensin I and isoprenaline was obtained beginning 10 min after administration of BW A575C.

Bolus intravenous injections of angiotensin I and isoprenaline (0.01–2 $\mu g\,kg^{-1}$) were administered in a constant volume of 1.0 ml kg^{-1} with an increasing sequence of dose.

Drugs used

BW A575C and enalaprilat were synthesized at the Wellcome Research Laboratories. Enalapril and captopril were generous gifts from Merck, Sharp and Dohme Ltd., and Squibb Ltd., respectively. Angiotensin I, isoprenaline and pindolol, were all obtained from Sigma Chemicals Ltd. BW A575C was prepared as a solution in either distilled water for *in vitro* studies or in 5% dextrose for *in vivo* studies.

Data analysis

The effects of angiotensin I or isoprenaline on diastolic blood pressure or heart rate respectively were expressed as absolute increases over control values. Results are expressed as mean ± s.e.mean where n is the number of animals. Linear regression analysis was used to fit straight lines to the linear part of the log dose-response relationship and the regression tested for parallelism. Providing there was no evidence of non-parallelism, dose-ratios were calculated and expressed with their 95% confidence limits.

The effects of isoprenaline in the guinea-pig atrial preparation were recorded as increases in atrial rate over basal. Concentration-effect curves obtained in this way were fitted to a logistic function in order to estimate ED_{50}s. These ED_{50}s were then analysed using the Schild equation in order to obtain pK_B values as described elsewhere (Black *et al.*, 1985). The fitting procedures were locally written, unweighted iterative least squares minimisation routines.

Results

ACE inhibition and β-adrenoceptor blocking studies in vitro

BW A575C (10^{-7}–10^{-5}M) produced a parallel rightwards shift of isoprenaline concentration-effect curves in guinea-pig right atria which was consistent with a simple competitive antagonism of the β-adrenoceptor. In order to quantify the antagonism, the ED_{50} values were analysed using the Schild equation and the slope of the Schild plot was found to be not significantly different from unity. The pK_B value obtained with the slope constrained to unity was determined to be 7.18 ± 0.05. Under identical experimental conditions a pK_B for pindolol was determined to be 8.9 ± 0.17 (Table 1). Neither BW A575C nor pindolol, over the concentration-ranges used, had any consistent effect upon basal heart rates of the preparation.

Addition of either enalaprilat or BW A575C to the enzyme reaction mixture of partially purified ACE from rabbit lung and its synthetic substrate (FA–PGG) inhibited in a time-dependent manner the

formation of the hydrolysis product. IC_{50} values calculated for both agents were 10.7 ± 2.1 nM and 4.4 ± 0.8 nM for BW A575C and enalaprilat, respectively (Table 1).

ACE inhibition and β-adrenoceptor blocking studies in vivo

Pithed rat studies Intravenous administration of angiotensin I ($0.01-10\,\mu g\,kg^{-1}$) to the pithed rat produced dose-dependent increases in diastolic blood pressure. Pretreatment with BW A575C ($1-100\,\mu g\,kg^{-1}min^{-1}$) inhibited in a dose-dependent fashion angiotensin I-induced pressor responses. At the lowest dose of BW A575C used ($1\,\mu g\,kg^{-1}min^{-1}$) a parallel rightwards displacement of the angiotensin I dose-response curve was obtained (dose-ratio with confidence limits, 3.6[2.64–4.98]). However, at the higher doses ($10-100\,\mu g\,kg^{-1}min^{-1}$), although a rightwards displacement of angiotensin I dose-response curves was obtained, loss of the maxima occurred, which did not permit quantitative regression analysis of the data (Figure 2). Low doses of BW A575C ($1\,\mu g\,kg^{-1}min^{-1}$) had no effect on resting diastolic blood pressure whereas the higher doses (10 and $100\,\mu g\,kg^{-1}min^{-1}$) produced a dose-dependent significant fall (10 ± 3 and 16 ± 3 mmHg respectively at these two doses) in resting diastolic blood pressure.

Intravenous administration of isoprenaline ($0.01-3\,\mu g\,kg^{-1}$) to the pithed rat produced a dose-dependent increase in heart rate which was inhibited dose-dependently by pretreatment with BW A575C ($10-100\,\mu g\,kg^{-1}\,min^{-1}$, Figure 2). At these doses, BW A575C did not significantly alter the resting heart rate of the pithed rat.

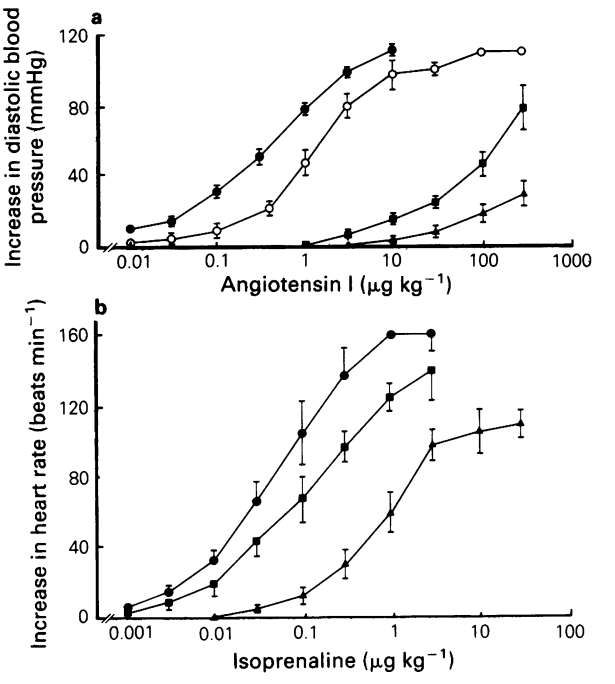

Figure 2 The effect of BW A575C ($1-100\,\mu g\,kg^{-1}min^{-1}$) on angiotensin I-induced pressor responses (a) and isoprenaline-induced tachycardia (b) in the pithed rat. Control dose-response curves (●) and those obtained after pretreatment with BW A575C $1\,\mu g\,kg^{-1}min^{-1}$ (○), $10\,\mu g\,kg^{-1}min^{-1}$ (■), $100\,\mu g\,kg^{-1}min^{-1}$ (▲), $n = 5-6$ for all groups.

Conscious rat studies Intravenous administration of angiotensin I ($0.003-3\,\mu g\,kg^{-1}$) to the conscious rat induced a dose-dependent increase in diastolic blood pressure and bradycardia. Pretreatment of conscious rats with either BW A575C ($1\,mg\,kg^{-1}$), enalapril ($1\,mg\,kg^{-1}$) or captopril ($10\,mg\,kg^{-1}$) caused a rightwards shift of the angiotensin I dose-response curve (Figure 3). In this respect BW A575C and enalapril were equipotent (dose-ratios with confidence limits were 29.4 [20.4–42.7] and 27.0 [18.6–39.2] for BW A575C and enalapril respectively) and both compounds were approximately ten times more potent than captopril. At this dose, BW A575C had a negligible effect on resting blood pressure and heart rate of the conscious rat.

A single intravenous injection of angiotensin I ($0.3\,\mu g\,kg^{-1}$) to the conscious rat caused a rise in diastolic blood pressure of 42.5 ± 1.0 mmHg which was consistent when repeated at 0.5 h intervals for up to 4 h in control animals. In groups of animals treated with BW A575C or enalapril at equieffective doses ($1\,mg\,kg^{-1}$) the pressor responses to administration of angiotensin I were markedly inhibited and full recovery to control responses was not attained until 4 h after treatment with the converting enzyme inhibitor (Figure 4).

Intravenous administration of isoprenaline ($0.03-3\,\mu g\,kg^{-1}$) to the conscious rat produced a dose-related

Table 1 pK_b values for BW A575C and pindolol, IC_{50} values for BW A575C and enalaprilat using assay conditions described in text for both properties

	ACE IC_{50} (nM)	*β-Blocking activity* pK_b
BW A575C	10.7 ± 2.1	7.18 ± 0.05
Enalaprilat	4.4 ± 0.8	—
Pindolol	—	8.9 ± 0.17

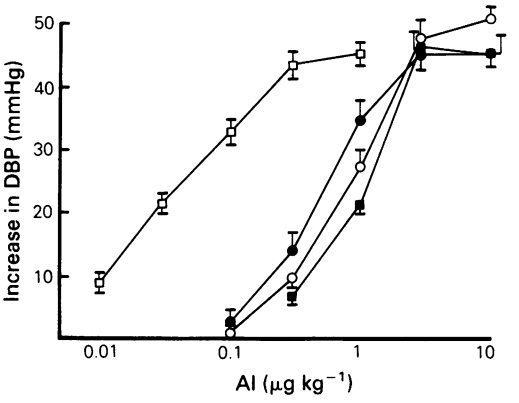

Figure 3 Effect of BW A575C (1 mg kg^{-1}, ○) enalapril, (1 mg kg^{-1}, ■) and captopril (10 mg kg^{-1}, ●) on control (□) angiotensin I-induced increases in diastolic blood pressure (DBP) in the conscious rat. $n = 6$ for all groups.

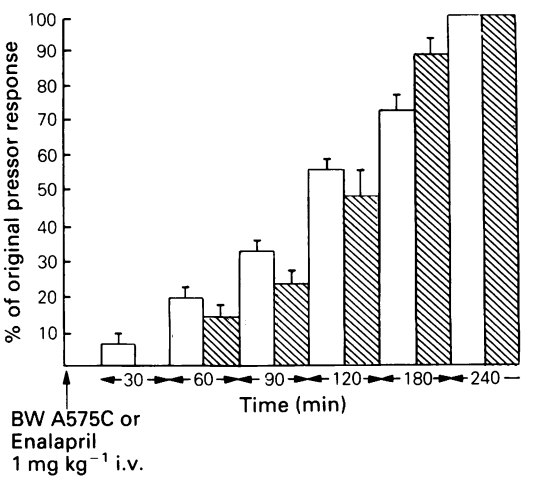

Figure 4 The effect of BW A575C (open columns) or enalapril (hatched columns) on the time course of recovery of a submaximal pressor response to angiotensin I (0.3 μg kg^{-1}) in the conscious rat. $n = 6$ for all groups.

tachycardia. Pretreatment with BW A575C (1 mg kg^{-1}) caused a rightwards shift of the isoprenaline dose-response curve and a dose-ratio (with confidence limits) of 3.1 (2.0–4.8) was obtained (Table 2).

A comparison of dose-ratios obtained for inhibition of angiotensin I-induced pressor responses and isoprenaline-induced tachycardia demonstrated that in this species BW A575C is approximately 10 times more active as an ACE inhibitor than as a β-adrenoceptor blocking agent (Table 2).

Conscious dog studies Intravenous administration of either angiotensin I or isoprenaline (0.01–2 μg kg^{-1}) to conscious dogs resulted in dose-related increases in diastolic blood pressure and heart rate, respectively (Figure 5). Pretreatment with BW A575C (0.5–1 mg kg^{-1}) caused a rightward shift of both angiotensin I and isoprenaline dose-response curves (Figure 5). Dose-ratios (with confidence limits) for inhibition of angiotensin I-induced pressor responses at the 0.5 and

Table 2 Dose-ratios for inhibition of angiotensin I-induced pressor responses (ACE) and isoprenaline-induced tachycardia (β) in conscious dogs and rats following pretreatment with BW A575C (1 mg kg^{-1})

	ACE	*β*	*ACE/β ratio*
Dog	15.9 (10.2–25.3)	8.1 (5.7–11.5)	1.96
Rat	29.5 (20–42.7)	3.1 (2.0–4.8)	9.5

Dose-ratios with 95% confidence limits are shown. $n = 5$–6 for both species.

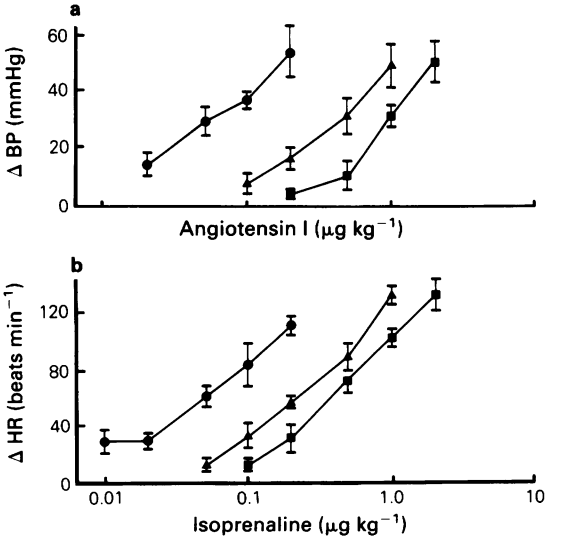

Figure 5 The effect of BW A575C (0.5–1 mg kg^{-1}) on angiotensin I-induced pressor responses (a) and isoprenaline-induced tachycardia (b) in the conscious dog. Control dose-response curves (●), after pretreatment with 0.5 mg kg^{-1} (▲), 1 mg kg^{-1} (■). $n = 5$ for all groups.

1 mg kg^{-1} doses were 6.8(4.6–9.8) and 15.9(10.2–25.3) respectively. Dose-ratios (with confidence limits) for inhibition of isoprenaline-induced tachycardia at the 0.5 and 1 mg kg^{-1} doses were 4.0(2.8–5.8) and 8.1(5.7–11.5) respectively. BW A575C, at these doses, had no effect on resting blood pressure and heart rate of the conscious dog. At both dose levels, dose-ratios obtained demonstrated that BW A575C was twice as potent as an ACE inhibitor than as a β-adrenoceptor blocking agent (Table 2).

Discussion

BW A575C is a chemically novel agent which was designed to incorporate both ACE-inhibition and β-adrenoceptor blocking properties into a single chemical entity. The data we have presented demonstrate that BW A575C exhibits both of the desired pharmacological properties and therefore represents the first novel chemical agent of this type displaying dual activity.

There is an extensive body of knowledge delineating structure-activity relationships of both β-adrenoceptor blocking agents and ACE inhibitors (Schier & Marker, 1981; Patchett & Cordes, 1985). Both classes of agents have broad overlapping structural requirements for activity, each being able to accept a range of aromatic systems to provide highly active compounds. However, when we attempted to hybridise β-adrenoceptor blocking agents based on a benzene (atenolol like) or naphthalene (propranolol like) nucleus with an ACE inhibitor of the enalapril type the resultant compounds failed to exhibit dual activity (Allan *et al.*, 1986a). However, we discovered that when the β-adrenoceptor blocking agent, pindolol and an ACE inhibitor of the enalapril type were used as templates for chemical hybridisation, the resultant chemical hybrid had dual activity (Allan *et al.*, 1986b). BW A575C is a chemical hybrid where the two active moieties are linked by a proteolytically stable secondary amide bond which is stable both *in vitro* and *in vivo* (unpublished data). The molecule contains four chiral centres, one in the side chain associated with β-adrenoceptor antagonist activity and the remainder in the peptide structure associated with ACE inhibition. The compound consists of a mixture of four diastereoisomers and therefore in common with other drugs that exist as diasteroisomeric mixtures the possibility exists that biological activity may reside within different isomers of the hybrid components. Nevertheless, the pharmacological activity of BW A575C both *in vitro* and *in vivo* demonstrates that it has retained potency and duration of action when compared to enalapril as an ACE inhibitor although potency as a β-adrenoceptor blocking agent has been reduced approximately 50 fold when compared to pindolol (assessed by *in vitro* studies only). Unlike pindolol, which has been reported to have appreciable partial agonist activity (Clark *et al.*, 1982) no evidence for partial agonism with BW A575C has been obtained. Our *in vivo* studies in conscious animals also demonstrate that BW A575C is approximately 2–10 fold more active as an ACE inhibitor than as a β-adrenoceptor blocking agent. The studies in conscious dogs (where the ratio between the two activities is as little as 2 fold) is particularly encouraging since it implies that both activities can be expressed *in vivo* within a narrow dose-range. Whether the balance of pharmacological activities of BW A575C is optimal to allow both properties to contribute in a mutually complementary manner to produce an antihypertensive action superior to an ACE inhibitor of β-adrenoceptor blocking agent alone remains to be evaluated.

Both ACE inhibitors and β-adrenoceptor blocking agents have been demonstrated to be very effective antihypertensive agents. Although it has been suggested that the most important action of these drugs is blockade of the renin angiotensin system albeit via different mechanisms (Buhler *et al.*, 1972; Case *et al.*, 1978) it is quite clear that β-adrenoceptor blocking agents can exert hypotensive actions independent of this system e.g. direct effects on the heart and central actions (Prichard & Owens, 1986). Thus a drug such as BW A575C with dual activity will not only be a potentially effective antihypertensive agent due to its ability to suppress the renin angiotensin system but will also demonstrate efficacy in hypertensive states where the cardiac response to physiological stimuli such as stress or exercise is controlled by β-adrenoceptor blockade (Ferguson *et al.*, 1986).

Although the antihypertensive actions of ACE inhibitors are well established, more recently it has been demonstrated that the ACE inhibitors, captopril and enalapril can alleviate both the subjective and objective evidence of cardiac failure and improve clinical status in congestive heart failure (Cleland *et al.*, 1984; Sharpe *et al.*, 1984). Although β-adrenoceptor blocking agents are generally contraindicated in patients with compromised left ventricular function, recent preliminary studies have demonstrated that β-adrenoceptor blocking agents may actually reduce the features of cardiac failure and improve prognosis in patients with chronic cardiac failure (Weber *et al.*, 1982). Therefore, an agent such as BW A575C which possesses potent ACE inhibitor and β-adrenoceptor blocking properties may also be useful in the treatment of congestive heart failure.

In conclusion, BW A575C displays dual activity as an ACE inhibitor with β-adrenoceptor blocking properties both *in vitro* and *in vivo* and therefore presents an exciting lead to the development of novel antihypertensive agents. Whilst there is a trend towards the design of dual active agents with com-

plementary biological activities, the development of true hybrid drugs has met with little success (Nicolaus, 1983). Whether BW A575C represents an example of a hybrid agent where two structure activity relationships and biological properties are not mutually exclusive and thereby proves to be a drug of clinical value awaits to be determined.

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