Mechanism of Action and Systemic and Regional Hemodynamics of the Potassium Channel Activator BRL34915 and Its Enantiomers

R.P. Hof, U. Quast, N.S. Cook, and S. Blarer

BRL34915 (BRL) is a vasodilator with a novel structure. Its mechanism of action, its effects on depolarization-induced and receptor-mediated blood vessel contraction, and its hemodynamic effects were investigated. In the rat portal vein, BRL inhibited spontaneous mechanical activity [IC₅₀ 0.013±0.001 μM (mean±SEM) for (−)-BRL], the initial effect being a reduced frequency of contraction. At higher concentrations, the spontaneous contractions were abolished and Rb⁺ efflux was increased. These results suggest that BRL preferentially acts on the pacemaker cells, the K⁺ channels in other cells being activated only at higher BRL concentrations in this vessel. In experiments on the rabbit aorta, (−)-BRL shifted the KCl concentration-response curve to the right and noncompetitively inhibited responses to angiotensin II. A concentration of 3 μM (−)-BRL reduced maximal angiotensin II contractions by around 50%, higher concentrations having little further effect. This inhibition of angiotensin II contractions is notably greater than that seen with Ca²⁺ antagonists in this vessel. In anesthetized rabbits, (−)-BRL was a peripheral vasodilator at doses of 3–30 μg/kg, but it had no relevant effects on heart rate and myocardial contractile force. This suggests tissue selectivity of this compound or this mechanism of action. BRL preferentially dilated the coronary, gastrointestinal, and cerebral vessels but not those of the kidneys or skeletal muscle as measured with tracer microspheres. This profile of activity is different from that of calcium antagonists or nonspecific vasodilators like dihydralazine. All effects were stereoselective, the (−)-enantiomer being 100 to 200 times more active than the (+)-enantiomer. (Circulation Research 1988;62:679–686)

Materials and Methods

Preparation of (−)-(3S,4R) and (+)-(3R,4S)-BRL34915

3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-(2-oxo-1-pyrolidinyl)-2H-1-benzopyran-6-carbonitrile was synthesized as follows: 3-bromo-3,4-dihydro-4-hydroxy-2,2-dimethyl-2H-1-benzopyran-6-carbonitrile was acetylated with (S)-camphanic acid chloride. The two resulting diastereoisomers were separated by column chromatography (Silicagel) and cleaved (NaOH, dioxane/water, 97%) to the corresponding epoxides. Epoxide ring opening yields the two optically active forms of BRL34915: (−)-(3S,4R) [α]D = −52.1° (c = 0.5, CHCl₃); and (+)-(3R,4S): [α]D = +51.9° (c = 0.5, CHCl₃). An 'H-NMR shift experiment [CDCl₃, (R)-(−)-1-(anthryl)-2,2,2-trifluoroethanol] showed the isomeric purity to be better than 97%.

Contraction of Rabbit Aortic Rings

Mongrel rabbits of either sex weighing 1.8–3.0 kg were killed by a sharp blow to the base of the skull. The descending thoracic aorta was excised immediately, cleaned of connective tissue, and aortic rings 2–3 mm wide were prepared and suspended in 10-mL organ baths containing a modified Krebs-Henseleit solution containing (mM) NaCl 118.0, KCl 4.7, MgSO₄ 1.2, CaCl₂ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25.0, EDTA 0.03, and glucose 10.1. The solution was maintained at 37°C and continually gassed with 5% CO₂ in oxygen. The tension
of the rings was recorded isometrically with electromechanical transducers (model UC3, Gould-Statham, Oxnard, California) coupled to an OKI if-800 Model 30 microcomputer (Oki Denki, Japan) capable of simultaneous recording from eight separate organ baths. At the beginning of the experiments, the rings were stretched to an initial tension of 1 g and allowed to equilibrate for approximately 1.5 hours, until a stable baseline of 100–200 mg tension was obtained.

During the equilibration periods, the bathing medium was changed at 15-minute intervals to prevent the accumulation of metabolites.

Cumulative concentration-response curves were obtained to either potassium chloride or angiotensin II. The maximum angiotensin II concentration was restricted to 10\(^{-7}\) M since it was found that higher concentrations failed to produce a greater steady-state contraction of the tissue and restricted the subsequent responsiveness of the tissue to angiotensin II. Rings were then washed and allowed to relax to baseline, the substance under investigation was added to the bath, and 30 minutes later the agonist concentration-response curves were repeated. The effects in the presence of substances were expressed as percentages of the preceding maximum response to the agonist (= 100%) for each aortic ring. Stock solutions of the enantiomers of BRL (10\(^{-2}\) M in ethanol:polyethylene glycol 400, 1:1) were freshly prepared each day and diluted further with Krebs-Henseleit solution as necessary. One of every four rings served as a control, receiving only the vehicle solution.

\(^{86}\)Rb\(^{+}\) Efflux and Myogenic Activity (Portal Veins)

Portal veins of freshly killed rats were incubated for 30 minutes in a HEPES-buffered physiological salt solution (PSS) gassed with 95% O\(_2\)-5% CO\(_2\) at 37\(^\circ\) C. The PSS contained (mM) NaCl 120, KCl 5, NaHCO\(_3\) 15, NaH\(_2\)PO\(_4\) 1.2, MgCl\(_2\) 2.5, CaCl\(_2\) 2.5, glucose 11, and HEPES 20, pH 7.4 at 37\(^\circ\) C. For loading with \(^{86}\)Rb\(^{+}\), the vein was incubated for an additional 90 minutes in PSS to which 5 \(\mu\)Ci/ml \(^{86}\)Rb\(^{+}\) had been added. The vein was then mounted in a thermostatic perfusion chamber similar to that described by Bolton and Clark. A preload of 500 mg was applied, and the chamber was perfused with PSS at 37\(^\circ\) C at a rate of 2.5 ml/min. The upper cotton thread was attached to an isometric force transducer (Gould cell, Statham) connected to a home-built amplifier from which the signal was given to a recorder and an integrator for quantitation of myogenic activity.

For measurement of \(^{86}\)Rb\(^{+}\) efflux, the perfusate was collected at a sampling rate of 2 minutes and counted for radioactivity in the Cerenkov mode at 50% efficiency. The radioactivity remaining in the portal vein at the end of the assay was determined by dissolving the vessel in 500 \(\mu\)l Lumasolve (Lumac, Schaeburg, The Netherlands) at 50\(^\circ\) C overnight. The sample was then supplemented with 500 \(\mu\)l 1 N HCl and 10 ml optifluor (Packard, Zürich, Switzerland) and counted in the \(^{3}P\)-channel at 100% efficiency.

The rate constant, \(k\), of \(^{86}\)Rb\(^{+}\) efflux was calculated as described by Quasi. Drug effects on the efflux rate constant were calculated as the peak value of \(k\) obtained during drug application divided by the basal value of \(k\) averaged over 6–10 minutes before drug application. Concentration-effect curves were fitted to a saturation hyperbola or to the Hill equation by nonlinear least-squares analysis. Errors in the parameters were estimated in the univariate approximation.

**Hemodynamic Experiments: Animals**

Large mongrel rabbits (body weight 3.5–4.5 kg) were anesthetized by injection into an ear vein of 25 mg/kg pentobarbital followed by 50 mg/kg phenobarbital 10–15 minutes later as described in detail elsewhere. The animals were tracheotomized and ventilated with a Loosco MK2 infant ventilator using room air. The ventilation was adjusted to keep the end-expiratory CO\(_2\) between 4.0 and 4.5 volume percent (measured continuously with a Gould-Godart capnograph). A positive end-expiratory pressure was applied as soon as the thorax was opened. Catheters were placed in the lower abdominal aorta, the inferior vena cava, and the jugular vein. The anesthesia was then deepened by a further 50 mg/kg phenobarbital. Through a thoracotomy in the left third intercostal space, the left atrium was cannulated for the injection of microspheres. The aortic root was cleaned of connective tissue, and a flow probe (Narco RT 500, Narco Bio-Systems, Houston, Texas; inner diameter 4.5–5.5 mm) was fitted on it. The electromagnetic flow probe was calibrated in vivo by the reference flow method at the time of the last microsphere injection. A second thoracotomy in the fifth right intercostal space was used to sew a Walton-Brodie strain-gauge onto the right ventricle parallel to the superficial muscle fibers.

**Microspheres**

We have described the use of the microsphere method previously in detail. In brief, for each measurement of regional blood flow about 1.5 \(\times\) 10\(^5\) microspheres were used, selected from various batches with one of the following labels: \(^{125}\)I, \(^{141}\)Ce, \(^{51}\)Cr, \(^{85}\)Sr, or \(^{46}\)Sc, as best suited. The spheres were injected into the left atrium with 1 ml of 0.9% saline. The reference sample was withdrawn from the lower abdominal aorta through the catheter in the femoral artery at a rate of approximately 6 ml/min.

At the end of the experiment, the animals were killed with an overdose of pentobarbital. The radioactivity of the samples was determined in a Packard gamma counter (model 5921) and the spectra processed on an OKI if-800 Model 30 microcomputer according to the method of Rudolph and Heymann with the modifications of the calculations described by Schosser et al.
Experimental Protocol

The racemic form and two enantiomers of BRL were dissolved in a vehicle containing ethanol 0.02 ml, polyethylene glycol 400, and glucose 5% 2 ml/mg of substance (total amount infused per kilogram body weight).

The same amount of vehicle alone was infused into a fourth group of animals (placebo group).

The effects of the two enantiomers were assessed using the Kruskal-Wallis test, and when it indicated a significant difference, this was followed by the Dunn-Bonferroni test to determine to which group the effect could be attributed.

Results

Effect of BRL34915 and Its Enantiomers on the Rat Portal Vein

Figure 2 shows the effect of the two enantiomers of BRL34915 on spontaneous activity and $^{86}\text{Rb}^+$ efflux in the rat portal vein in representative experiments. In the left panel, superfusion of the vessel with 0.03 $\mu$M of the active enantiomer, (−)-BRL, for 20 minutes is seen to drastically diminish spontaneous activity by decreasing the frequency of the spontaneous contractions while leaving their amplitude almost unaltered. The rate constant, $k$, of $^{86}\text{Rb}^+$ efflux was not affected. The effect of (−)-BRL was readily washed out, and application of 0.3 $\mu$M (−)-BRL led to complete mechanical quiescence accompanied by an increase in the flux rate constant of 24%. After a washout phase of 30 minutes, a concentration of 30 $\mu$M (−)-BRL was applied. After having reached a peak value of 165%, the efflux rate constant decreased again despite the continued presence of (−)-BRL. This decrease probably reflects inactivation of the response (channel? See Quast et al.).

The effects with (+)-BRL are shown in the right panel of Figure 2. Concentrations at least 100 times higher were needed to produce effects similar to those seen with (−)-BRL. At 3 $\mu$M, (+)-BRL depressed spontaneous activity by 55%, again reducing the frequency but not the height of the contractile spikes. The rate constant of $^{86}\text{Rb}^+$ flux was minimally affected. At 100 $\mu$M, (+)-BRL inhibited spontaneous activity completely and increased the flux rate constant by 22%.

In this case, washout of the substance was followed by an overshoot of mechanical activity. The racemic mixture gave similar results to those obtained with the active enantiomer (not shown).

The concentration dependence of the effects of BRL and its enantiomers on $^{86}\text{Rb}^+$ efflux is presented in Figure 3. Inhibition of spontaneous activity (left panel) gave IC$_{50}$ values of 0.013 ± 0.001 $\mu$M for (−)-BRL and 2.2 ± 0.4 $\mu$M for (+)-BRL, separated thus by a factor of 170 (mean ± SEM). The IC$_{50}$ value for the racemate (0.024 ± 0.005 $\mu$M) is about the theoretical factor of two higher than that of the active enantiomer. The concentration-effect curves were steep, as indicated by the Hill coefficients of approximately 1.5.

The concentration dependencies of the (−)-enantiomer and of the racemate were bell-shaped as the peak response decreased again at 100 $\mu$M, the highest concentration used. This decrease probably reflects the desensitization process mentioned above, which may prevent full development of the effect. Leaving out the point at 100 $\mu$M allows a fit of the remaining data to a saturation hyperbola, which gives midpoints of 1.7 ± 0.3 $\mu$M for (−)-BRL and 2.5 ± 0.5 $\mu$M for (+)-BRL with theoretical saturation values of 1.6 ± 0.2 for (−)-BRL, 1.4 ± 0.3 for (+)-BRL, and 1.4 ± 0.4 for (+)-BRL. The respective IC$_{50}$ values are 0.013 ± 0.001 $\mu$M, 2.2 ± 0.4 $\mu$M, and 0.024 ± 0.005 $\mu$M.
131 ± 6% and 116 ± 5%, respectively. The apparent saturation behavior of the flux response may well be due to the occurrence of desensitization and need not reflect saturation of agonist binding.

It should be noted that stimulation of Rb⁺ efflux is observed only at relatively high concentrations; 10% stimulation of efflux occurs at concentrations about 10 times those necessary to inhibit spontaneous activity, and the midpoints of the flux curves occur at concentrations 100 times higher than the IC₅₀ values for spontaneous activity (see Figure 3). As with the inhibition of spontaneous activity, the stimulation of Rb⁺ efflux by BRL was also stereoselective, the activity residing in the (-)-enantiomer.

**Inhibition of Contraction of Rabbit Aorta**

In rabbit aortic rings contracted by potassium chloride depolarization, concentrations of 0.01 and 1.0 µM (-)-BRL caused a small rightward shift of the potassium chloride concentration-response curve (Figure 4, upper panel). Contractions due to potassium chloride concentrations up to 32 mM were diminished in the presence of (-)-BRL, but contractions due to higher potassium chloride concentrations were little affected. At the higher concentration of 100 µM (-)-BRL, there was also evidence of a reduction in the maximum tissue response to potassium chloride. With the (+)-enantiomer of BRL (Figure 5, upper), concentrations of 1 and 10 µM had no effect on the responses to potassium chloride and only at 100 µM was there some evidence of a rightward shift of the potassium chloride concentration-response curve, with a small reduction in the maximum tissue response to potassium chloride. This diminution of the maximal response of aortic rings to depolarization-induced contraction by 100 µM concentrations of both enantiomers of BRL thus appears to lack the stereoselectivity that characterizes the other actions of this drug, possibly indicating the existence of nonspecific effects at these high concentrations.

Aortic rings contracted by receptor stimulation with angiotensin II revealed a quite different antagonistic profile of BRL. The (-)-enantiomer potently antagonized angiotensin II responses, resulting in a non-competitive inhibition of the angiotensin II concentration-response curve (Figure 4, lower). At a concentration of 3 µM, (-)-BRL inhibited contrac-
vasodilators. The large increase in total peripheral conductance induced by BRL indicates that this agent is a peripheral vasodilator. This dilatation was, however, not generalized, but showed instead selectivities for some vascular beds. Figures 7A and 7B show the regional distribution of the peripheral vascular effects expressed as changes in regional conductance. BRL in all its forms caused coronary vasodilatation (Figure 7A), which tended to be more pronounced in the outer layer of the left ventricular free wall than in the subendocardial layer.

Figure 7B shows further organs where BRL and its enantiomers elicited vasodilatation. In the brain, this effect was weak, reaching significance only at the highest dose of the racemic mixture and the (−)-enantiomer. Vasodilatation was pronounced in the stomach, where the effect reached significance at the two higher doses of each compound and in the case of the (−)-enantiomer at all three doses used. A somewhat weaker vasodilatation was seen in the small intestine, where the effects were again dose-related but significant only at the highest dose of the three forms of the compound. Finally, no relevant vasodilatation was found in the kidney and in skeletal muscle.

### Discussion

BRL and its enantiomers are peripheral vasodilators. Their systemic hemodynamic, but not their regional effects, were qualitatively similar to those of vasoselective calcium antagonists. The mechanisms of action of calcium antagonists and potassium channel activators resemble each other in that both are assumed to reduce calcium influx into cells, calcium antagonists by directly interfering with the opening of slow L-type calcium channels and K⁺-channel activators by hyperpolarizing the cell membrane and thus opposing the opening of such voltage-dependent Ca²⁺ channels. The present study shows that striking differences exist in the profiles of action of these two classes of vasodilators both in vitro and in vivo. First, we wish to consider

### Experimental Animals: Systemic Hemodynamic Effects

The baseline values of the four groups before drug administration (Table 1) were comparable (Kruskal-Wallis test). BRL and its enantiomers induced a fall in blood pressure as shown in Figure 6. The racemic mixture was about half as active as the pure (−)-enantiomer, and the (+)-enantiomer was more than 100 times less active. Heart rate showed a weak tendency to increase with the racemic mixture and the less active enantiomer, and it increased significantly with the (−)-enantiomer (9% at the highest dose). Myocardial contractile force, measured directly with a strain gauge, remained unchanged as compared with placebo. Cardiac output increased dose-dependently. The effect reached statistical significance at the highest doses of both enantiomers. The increases in total peripheral conductance were more pronounced and reached statistical significance with the highest doses of each compound, as compared with placebo-treated animals.

### Regional Circulation

The large increase in total peripheral conductance induced by BRL indicates that this agent is a peripheral vasodilator. This dilatation was, however, not generalized, but showed instead selectivities for some vascular beds. Figures 7A and 7B show the regional distribution of the peripheral vascular effects expressed as changes in regional conductance. BRL in all its forms caused coronary vasodilatation (Figure 7A), which tended to be more pronounced in the outer layer of the left ventricular free wall than in the subendocardial layer.

### Table 1. Baseline Values for Hemodynamic Effects

<table>
<thead>
<tr>
<th>Baseline Values</th>
<th>Control</th>
<th>(+)</th>
<th>(−)</th>
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</thead>
<tbody>
<tr>
<td><strong>Systemic variables</strong></td>
<td></td>
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</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>80 ± 3.37</td>
<td>68 ± 3.29</td>
<td>74 ± 1.95</td>
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<tr>
<td>HR (beats/min)</td>
<td>296 ± 8.29</td>
<td>285 ± 21.0</td>
<td>258 ± 7.56</td>
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<tr>
<td>CVP (mm Hg)</td>
<td>2.97 ± 0.52</td>
<td>2.97 ± 0.45</td>
<td>3.47 ± 0.74</td>
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<tr>
<td>CF (g)</td>
<td>26 ± 1.04</td>
<td>27 ± 4.05</td>
<td>29 ± 1.69</td>
</tr>
<tr>
<td>CO (ml/min/kg)</td>
<td>92 ± 10.23</td>
<td>90 ± 6.24</td>
<td>94 ± 5.23</td>
</tr>
<tr>
<td>TPC (ml/min/mm Hg/kg)</td>
<td>1.16 ± 0.17</td>
<td>1.32 ± 0.03</td>
<td>1.27 ± 0.06</td>
</tr>
<tr>
<td><strong>Regional conductance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart total</td>
<td>2.02 ± 0.123</td>
<td>2.33 ± 0.109</td>
<td>2.26 ± 0.099</td>
</tr>
<tr>
<td>Epi</td>
<td>2.53 ± 0.273</td>
<td>3.11 ± 0.208</td>
<td>3.18 ± 0.162</td>
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<tr>
<td>Mid</td>
<td>3.02 ± 0.251</td>
<td>2.79 ± 0.183</td>
<td>2.94 ± 0.146</td>
</tr>
<tr>
<td>Endo</td>
<td>2.96 ± 0.181</td>
<td>2.89 ± 0.097</td>
<td>3.09 ± 0.198</td>
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<tr>
<td>Brain</td>
<td>0.509 ± 0.062</td>
<td>0.552 ± 0.049</td>
<td>0.597 ± 0.023</td>
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<tr>
<td>Kidneys</td>
<td>3.54 ± 0.770</td>
<td>4.18 ± 0.445</td>
<td>4.20 ± 0.355</td>
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<tr>
<td>Muscle</td>
<td>0.075 ± 0.027</td>
<td>0.068 ± 0.018</td>
<td>0.045 ± 0.003</td>
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<tr>
<td>Stomach</td>
<td>0.796 ± 0.247</td>
<td>0.826 ± 0.216</td>
<td>0.754 ± 0.110</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.624 ± 0.090</td>
<td>0.627 ± 0.132</td>
<td>0.449 ± 0.030</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; CVP, central venous pressure; CF, contractile force; CO, cardiac output; TPC, total peripheral conductance.
FIGURE 6. Systemic hemodynamic effects of the infusion of BRL. Cumulative doses of 3, 10, and 30 μg/kg of the racemate (±), 0.3, 1, and 3 mg/kg of the (+)-enantiomer and 3, 10, and 30 μg/kg of the (−)-enantiomer were infused. C indicates spontaneous changes observed in a control group infused with the vehicle of the active agents. Effects are shown as percent changes from the baseline values listed in Table 1. Bars show the SEM, n = 5.

*Significant differences from the vehicle-treated animals (Kruskal-Wallis and Dunn-Bonferroni tests, p<0.05).

HR, heart rate (beats/min); MAP, mean arterial pressure (mm Hg); CF, contractile force (gram); CVP, central venous pressure (mm Hg); CO, cardiac output (ml/min/kg); TPC, total peripheral conductance (ml/min/mm Hg/kg).

Experimental evidence that BRL acts indeed as a K⁺-channel activator.

Effects of BRL on Rat Portal Vein: Evidence for Increased K⁺ Permeability

At concentrations above 0.1 μM, BRL stimulated ⁸⁶Rb⁺ efflux from the portal vein (Figure 2), which reflects an increase in the K⁺ permeability of the smooth muscle membrane. This activity of BRL resided primarily in the (−)-enantiomer, with the (+)-enantiomer increasing flux only at concentrations 100 times higher. Additional support for the notion that BRL acts by increasing the cell membrane K⁺ permeability stems from the particular pattern by which spontaneous activity of the portal vein was inhibited. As shown in Figure 2, BRL first decreased the frequency of the contractions without reducing their amplitude. This is typical of hyperpolarization of a pacemaker, by activation of K⁺ channels after a burst of activity. Two other vasodilators now known to act like BRL via K⁺-channel activation, nicorandil and pinacidil, also decrease spontaneous activity of the portal vein at concentrations 10 times lower than those needed to cause ⁸⁶Rb⁺ efflux. This, together with the stereoselectivity of BRL's effects, further implicates the activation of membrane K⁺ channels in the vasodilator action of BRL.

Profile of Activity on Rabbit Aortic Rings

In rabbit aortic rings contracted by potassium chloride depolarization, calcium channel antagonists are known to noncompetitively inhibit the potassium chloride concentration-response curves but have relatively little effect on receptor-mediated contractions. A preferential action of BRL on the pacemaker regions of the portal vein (perhaps due to the particular sliding membrane potential there) would also explain why stimulation of ⁸⁶Rb⁺ efflux is observed only at concentrations higher than those required to decrease the frequency of spontaneous activity. If low concentrations of BRL primarily increase the K⁺ permeability of the pacemaker cells, this will inhibit myogenic activity by decreasing the frequency without producing a measurable increase in overall ⁸⁶Rb⁺ efflux, since pacemaker cells are scarce. The bulk of the smooth muscle cells presumably responds only at higher BRL concentrations, leading to the observed increase in ⁸⁶Rb⁺ flux. Moreover, spiking activity itself causes efflux of ⁸⁶Rb⁺, and the decreased frequency after BRL probably masks the increases elicited by BRL. If spike generation in the portal vein is prevented by addition of a Ca²⁺ antagonist, then BRL-stimulated ⁸⁶Rb⁺ efflux can be detected from concentrations as low as 60 nM, which is more in line with the concentrations that reduce spontaneous activity in this vessel. Two other vasodilators now known to act like BRL via K⁺-channel activation, nicorandil and pinacidil, also decrease spontaneous activity of the portal vein at concentrations 10 times lower than those needed to cause ⁸⁶Rb⁺ efflux. This, together with the stereoselectivity of BRL's effects, further implicates the activation of membrane K⁺ channels in the vasodilator action of BRL.
However, BRL noncompetitively inhibited angiotensin II contractions of the aorta but caused only a small rightwards shift of the concentration-response curve to potassium chloride. In both cases, the activity appeared to reside primarily in the (−)-enantiomer, with the (+)-enantiomer being approximately 100 times less active. This apparent rightward shift of the potassium chloride curve by BRL, which has been found previously in the rat aorta and portal vein, is what one might expect as a consequence of K+-channel activation causing membrane hyperpolarization, which functionally antagonizes the depolarization elicited by potassium chloride. The inability of BRL to inhibit contractions by high concentrations of potassium chloride is indeed predictable from the Nernst equation. These high potassium chloride concentrations shift the potassium equilibrium potential towards more positive membrane potentials. Hence, when the bath potassium chloride concentration exceeds about 32 mM (dependent on the intracellular K+ concentration and the resting membrane potential of the aorta), the membrane potential will always be above the threshold potential for opening of voltage-sensitive Ca2+ channels (around −40 mV), with the consequence that K+-channel activating drugs should no longer be able to modulate the opening of the Ca2+ channels (see also Hamilton et al2). Only at 100 μM was any effect of BRL (or its enantiomers) elicited against these contractions by high potassium chloride concentrations, the lack of stereoselectivity at this concentration suggesting it to be a nonspecific effect.

A 50% inhibition of angiotensin II contractions was seen with 3 μM (−)-BRL. Higher concentrations were found to cause little further reduction of the angiotensin II responses (Figure 4, lower panel). The underlying mechanism is not known. Extracellular Ca2+ plays an important role in the contraction of this blood vessel to angiotensin II. However, organic Ca2+ antagonists inhibit only a small component of the angiotensin II response in rabbit aorta. Therefore, indirect inhibition of Ca2+ entry through classical (L-type) voltage-sensitive Ca2+ channels cannot explain the inhibitory action of the K+-channel activators against angiotensin II contraction of the rabbit aorta.

It is not known to what extent alterations of the cell membrane potential might influence the release of intracellularly stored Ca2+. Whether the part of the angiotensin II response antagonized by BRL is due solely to inhibition of Ca2+ influx has still to be established.

### Hemodynamic Effects in Whole Animals

The systemic vascular effects of BRL and its enantiomers are qualitatively similar to those of other vasodilators such as hydralazine or, perhaps more appropriately, those of vasoselective calcium antagonists. They clearly differed from those of calcium antagonists with strong cardiac effects such as verapamil or diltiazem. This is an indication that the mechanism of action of BRL shows a certain degree of selectivity for K+ channels in vascular smooth muscle over those in the heart as has been shown in a preliminary report in vitro. Major differences between BRL and its enantiomers and these other vasodilators were found, however, with respect to the peripheral vascular regions studied. A relatively vasoselective calcium antagonist, darodipine, has been examined in the same experimental model so that a direct comparison is possible. Darodipine increased coronary and especially cerebral blood flow much more than BRL at doses causing comparable falls in blood pressure. Calcium antagonists, especially dihydropyridines, but not BRL, strongly dilate skeletal muscle vessels. By contrast, BRL dilated the gastrointestinal vascular bed, which is affected little by calcium antagonists. Only in the rabbit, but not in other species (discussed in Hof et al), dihydropyridines cause vasoconstriction in the kidney, whereas BRL was inactive in this vascular bed. In cats, BRL has been found to increase renal flow, whereas calcium antagonists cause little or no change. This looks similar to, but probably is not, autoregulation since these agents have been shown to interfere with renal autoregulation. It is perhaps more appropriate to say that the vasodilator effects of calcium antagonists are of sufficient magnitude to compensate for the simultaneously occurring fall in blood pressure.

It is interesting to note that in cats, the effects of BRL on other vascular beds (femoral and mesenteric blood flow measured with electromagnetic flowmeters) were similar to those we observed in the rabbit experiments. Interestingly, dihydralazine dilated the same vascular beds as calcium antagonists, thus presenting a hemodynamic profile of activity different from that of BRL.

In conclusion, all effects of BRL examined in our experiments were stereoselective. Our results do not suggest a separate biological activity for the (−)-enantiomer and are compatible with a very small (0.5–1.0%) admixture of the (−)-enantiomer. The results suggest that BRL to evoke 86Rb+ efflux from this tissue but also demonstrate effects of BRL (and its enantiomers) on mechanical activity at concentrations below those at which 86Rb+ efflux could be detected. Additionally, in the rabbit aorta, the ability of these compounds to attenuate angiotensin II contractile responses could not be explained merely in terms of an indirect inhibition of Ca2+ entry through dihydropyridine-sensitive Ca2+ channels. The in vivo results also demonstrate major differences in the vasodilator profiles of BRL and Ca2+ antagonists, suggesting once again that this class of drugs should not be regarded merely as indirect-acting Ca2+ antagonists. It will be interesting to see how these results extrapolate to the clinical situation where such compounds with a new specific mechanism of action could offer exciting new insights into the human pathophysiology of the cardiovascular system.

### References

1. Buckingham RE, Clapham JC, Hamilton TC, Longman SD, Norton J, Poyser RH: BRL34915, a novel antihypertensive agent: Comparison of effects on blood pressure and other...


37. Key Words: *BRL34915* • enantiomers • potassium channels • *$[^{38}Rb]$* efflux • haemodynamic effects • microspheres
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