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SPECIFICITY OF ACTION OF THE NOVEL ANTIHYPERTENSIVE AGENT, BRL 34915, AS A POTASSIUM CHANNEL ACTIVATOR

COMPARISON WITH NICORANDIL

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Abstract—Experiments have been performed to investigate the specificity of the mechanism of action of the novel antihypertensive agent, BRL 34915. BRL 34915 (0.5–100 μM) and nicorandil (10–500 μM) stimulated the efflux of rubidium from preloaded rabbit isolated mesenteric arteries. BRL 34915 also caused an increase in the rubidium efflux rate constant in other vascular smooth muscles. Tetraethylammonium (0.1–30 mM) inhibited BRL 34915 (10 μM), nicorandil (100 μM) and potassium (30 mM) induced stimulations of rubidium efflux, but had no effect on noradrenaline (30 μM) induced efflux. Only noradrenaline induced efflux was inhibited by apamin (3–100 nM). Examination of other second messenger systems demonstrated that BRL 34915 (at concentrations up to 100 μM) did not have any appreciable effect on cGMP accumulation in rabbit mesenteric artery, cAMP or cGMP phosphodiesterase in rat heart, or cAMP and inositol phosphate accumulation in rat brain slices. Nicorandil (100 μM) caused a small increase in cGMP accumulation in rabbit mesenteric artery. Radioligand binding studies showed that BRL 34915 did not interact with dihydropyridine, 5-hydroxytryptamine, dopamine, α_1, α_2 or β adrenoceptor binding sites. [^{3}H]-BRL 34915 did not bind specifically to any site in any tissue studied, either *in vitro* or *ex vivo*. Thus we have been unable to demonstrate an effect of BRL 34915 other than of increasing potassium efflux in rabbit vascular smooth muscle. This lends support to other evidence suggesting that BRL 34915 relaxes vascular smooth muscle (and hence lowers blood pressure) by a novel, and specific, mechanism involving hyperpolarisation of the smooth muscle cell membrane.

BRL 34915, (±)6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2*H*-benzo[*b*]-pyran-3-ol, is a member of a series of structurally novel benzopyrans [1] which acts on the vasculature to lower blood pressure in man [2] and several animal species [3]. It has been proposed that BRL 34915 exerts this effect by virtue of its ability to impair contractions by hyperpolarising the vascular smooth muscle membrane [4]. Evidence to support this hypothesis comes from studies in which BRL 34915 increased rubidium efflux from preloaded rabbit isolated mesenteric artery [5], rat portal vein [4] and rat aorta [6]. Furthermore, these effects have been demonstrated in other smooth muscles, such as guinea-pig taenia caeci [7] and trachealis [8]. However, inhibition of spontaneous electrical discharges in rat portal vein occurred at concentrations of BRL 34915 which did not produce detectable hyperpolarisation [4].

It has been reported that the anti-anginal agent nicorandil [9] opens potassium channels in vascular [10, 11] and other [12] smooth muscles, and increases 86-rubidium efflux in a variety of smooth muscles [6, 7, 13]. However, it has also been suggested that some of the effects of nicorandil are mediated by an increase in cGMP levels which is associated with the presence of a nitro group within the molecule [14, 15].

The relaxant effects of nicorandil would appear to be due to at least two mechanisms, one of which is shared with BRL 34915. Moreover, in rat portal vein inhibition of electrical activity by BRL 34915 occurs without detectable hyperpolarisation. We have, therefore, further studied the effect of BRL 34915 on rubidium efflux and examined the specificity of its action in a variety of other systems.

MATERIALS AND METHODS

Materials

[^{3}H]-Cyclic AMP, [^{3}H]-cyclic GMP, [^{3}H]-5-hydroxytryptamine, [^{3}H]-prazosin, [^{3}H]-RX 781094, [^{3}H]-spiperone and [^{3}H]-cyclic AMP radioimmunoassay kits were obtained from Amersham International. [^{3}H]-Dihydroalprenolol, [^{3}H]-domperidone, [^{3}H]-myoinositol, [^{3}H]-nitrendipine, ^{86}RbCl and [^{125}I]-cyclic GMP radioimmunoassay kits were obtained from New England Nuclear. [^{3}H]-BRL 34915 was prepared by custom synthesis and catalytic tritiation (Amersham International) and by Dr K. Willcocks of Beecham Pharmaceuticals, Harlow; the specific activity was 25.9 Ci/mmole. Tetraethylammonium chloride, noradrenaline bitartrate, apamin, acetylcholine and papaverine were supplied by Sigma. Sodium nitroprusside was supplied by BDH and 5-hydroxytryptamine creatinine sulphate by May and Baker. We thank the following

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companies for gifts of the appropriate drugs: Chugai (nicorandil), Bayer (nifedipine and nitrendipine), Hassle (felodipin), Ciba Geigy (hydrallazine), Janssen (spiperone), and Pfizer (prazosin). RX 781094 (idazoxan) was prepared by Mr C. Johnson of Beecham Pharmaceuticals, Harlow.

Methods

86-Rubidium efflux. Sections of mesenteric, pulmonary, brachial and ear arteries, or abdominal aorta, from male New Zealand White rabbits were removed and immediately placed in aerated (95/5% O_2/CO_2) HEPES buffer at 37° (composition in mM: NaCl 120; KCl 6.0; $CaCl_2$ 2.5; $MgCl_2$ 1.2; HEPES 5.0; glucose 11.4; pH 7.4). Arteries were cleaned of connective tissue, opened lengthwise and cut into segments of 10–20 mg wet weight. Individual pieces of artery, fixed on small stainless steel hooks, were suspended in a tissue bath containing about 200 ml of buffer (37°, slow aeration). After 30 min, 100–200 μCi 86-rubidium (1–6 mCi/mg) was added to the bath and tissues left to equilibrate for 90 min. Each tissue (still attached to its hook) was then transferred, at 3 min intervals, through a series of plastic vials containing 3 ml fresh, aerated HEPES buffer (but no radioactivity). During this time the vials were agitated gently in a shaking water bath at 37°. The ability of compounds to enhance efflux was tested by exposing the tissue to the drug under test between minutes 30 and 45 of the efflux period (1 hr). Substances tested for their ability to inhibit stimulation of efflux were present throughout the whole efflux period. Radioactive content of the vials was determined by liquid scintillation counting, tissues being weighed and solubilised in 1 ml Soluene (Packard Instruments) prior to counting.

Results are expressed as rate coefficients which were calculated as the 86-rubidium released (counts) during each 3 min period as a percentage of the mean tissue 86-rubidium remaining during that period. The mean rate coefficient over minutes 21–30 of the efflux period was taken as the basal rate. Drug stimulation of efflux rate was calculated as the maximum efflux rate observed over minutes 30–45 of the efflux period divided by basal rate and was expressed as a percentage.

Cyclic GMP accumulation. Individual pieces of mesenteric artery were prepared as described in the previous section and suspended in 10 ml organ baths containing Krebs Henseleit buffer (composition in mM: NaCl 118; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25; glucose 10.1; pH 7.4) at 37° and gassed with 95/5% O_2/CO_2. An equilibration period of 1 hr was allowed, with several changes of buffer. At the end of this time the drug under test was added to the bath and, following a 1.5 min exposure period, the tissue was frozen with tongs precooled in liquid nitrogen. The tissues were stored at −70° (<48 hr) until assayed for cyclic GMP. The extraction and acetylated radioimmunoassay of cyclic GMP followed closely the instruction of the manufacturer (New England Nuclear), with the exception that tissues were homogenised in a microdismembrator (Braun Melsungen A.G.) using Teflon capsules and tungsten carbide balls precooled in liquid nitrogen. Protein concentrations were measured according to the method of Lowry *et al.* [16].

Cyclic AMP accumulation. The accumulation of cyclic AMP in rat cortical slices was studied using a method based on that described by Forn *et al.* [17], with cyclic AMP content being determined using a cyclic AMP radioimmunoassay kit.

Rat heart cyclic AMP and cyclic GMP phosphodiesterase (PDE) activity. Cyclic AMP and cyclic GMP PDE activity was measured in rat heart membranes by a method based on Rutten *et al.* [18] with modifications from Hidaka and Shibuya [19].

Inositol phosphate turnover. The hydrolysis of phosphatidylinositol was assayed by a method based on that of Berridge *et al.* [20].

Radioligand binding studies. Hearts from male Sprague–Dawley rats were homogenised in 5 vol. of 50 mM Tris–HCl, pH 7.4. The 50,000 *g* (4°) pellet from two hearts was washed 3 times in fresh buffer before being resuspended in 10 ml and kept on ice until use for [^{3}H]-nitrendipine binding. Brain areas of male Sprague–Dawley rats were homogenised in 5 vol. of 50 mM Tris–HCl, pH 7.4. The pellet was washed and resuspended as described for [^{3}H]-nitrendipine binding. The sites labelled were $5HT_1$ and $5HT_2$ in frontal cortex (using [^{3}H]-5HT and [^{3}H]-spiperone), α_1, α_2 and β-adrenoceptors in cortex (using [^{3}H]-prazosin, [^{3}H]-RX 781094 and [^{3}H]-dihydroalprenolol) and dopamine receptors in corpus striatum ([^{3}H]-domperidone). Samples were rapidly filtered under vacuum through Whatman GF/B filters with 3 × 4 ml washes. The radioactivity bound to membranes trapped by the filters was determined by liquid scintillation counting.

[^{3}H]-*BRL 34915 binding*. Tissues were removed from male Sprague–Dawley rats and homogenised in a variety of buffers: 50 mM Tris HCl, pH 7.4; 50 mM Tris–100 mM NaCl, pH 7.4; 50 mM Tris–1 mM EGTA, pH 7.4; 0.32 M sucrose; 10 mM HEPES–1 mM phenanthroline, pH 7.4; Krebs–Henseleit. Crude homogenates or the pellet or supernatant from 50,000 *g* centrifugation procedures were examined. Furthermore, tissue slices, chopped on a McIlwain slicer, were also used in some experiments. The following tissues/organs were used in these studies: heart, kidney, adrenal gland, spleen, lung, liver, aorta, mesenteric bed, renal artery, portal vein, plasma, platelets, red blood cells, whole brain and brain areas. Tissues were either prepared fresh on the day of use or frozen until assayed.

The tissue preparations described above were incubated with [^{3}H]-BRL 34915 with various changes in the assay conditions e.g. buffer, ions, temperature, time and pH, the addition of protease inhibitors and bovine serum albumin. In all experiments, half the assay tubes contained 1 μM BRL 34915 (unlabelled) to indicate the presence of any displaceable binding.

Statistical analysis. Statistical analysis was carried out using Student's *t*-test. An effect was considered significant when $P < 0.05$.

RESULTS

86-Rubidium efflux

The basal efflux rate in rabbit isolated mesenteric artery was found to average about 2–2.5% per 3 min

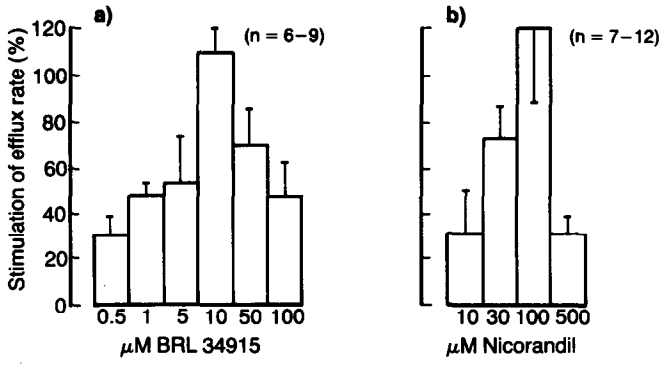

Fig. 1. Stimulation of 86-rubidium efflux from rabbit isolated mesenteric artery by (a) BRL 34915 and (b) nicorandil. Stimulation of efflux rate, at the indicated BRL 34915 or nicorandil concentration, is the maximum efflux rate observed during exposure to the drug divided by basal efflux rate and is presented as mean with standard error bar from number of experiments shown in parenthesis.

efflux period (which is approximately 5–30 fmol rubidium released per min per mg wet weight). BRL 34915 (0.5–10 μM) produced a concentration related increase in 86-rubidium efflux from rabbit isolated mesenteric artery, with the maximum stimulation produced by BRL 34915 occurring at 10 μM. Higher concentrations of BRL 34915 produced less stimulation of efflux (Fig. 1)—the reason for this is unknown. The stimulation caused by BRL 34915 was not maintained throughout the exposure time (18 min) but slowly began to return to basal levels (Fig. 2). Nicorandil induced efflux also showed a bell-shaped concentration response curve, with the maximum effect at 100 μM (Fig. 1), and, like BRL 34915, the maximal increase in efflux rate constant was not maintained throughout the time of exposure to the drug. The maximum increases in 86-rubidium efflux produced by these two compounds were similar to those observed with 30 μM noradrenaline or 30 mM potassium (see e.g. Fig. 3).

In a separate series of experiments it was shown that the basal efflux rates and maximum stimulation of efflux rates caused by BRL 34915 were approximately the same in pulmonary, ear and brachial arteries, and abdominal aorta (Table 1). The concentration of BRL 34915 required to cause maximal stimulation lay within a ten fold range (1–10 μM).

The stimulation of efflux from rabbit isolated mesenteric artery by BRL 34915 (10 μM) and nicorandil (100 μM) was inhibited in a concentration dependent manner by tetraethylammonium (TEA) (1–30 mM) (Fig. 3). Potassium (30 mM) induced rubidium efflux was also significantly reduced by TEA in a concentration dependent manner, but, at the concentrations used, TEA did not completely abolish the response. There was no statistically significant effect of TEA on noradrenaline (30 μM) stimulated efflux (Fig. 3). TEA (30 mM) had no effect on basal efflux rate (2.35 ± 0.26% per 3 min in the presence of TEA (N = 8); 2.23 ± 0.23% per 3 min in the absence of TEA (N = 6)).

The bee venom toxin, apamin (3–100 nM) caused a reduction in noradrenaline induced efflux (Fig. 4) with a maximum significant inhibition of approximately 40% at 100 nM. The highest concentration of apamin (100 nM) had no effect on basal efflux rate (2.68 ± 0.28% per 3 min in the presence of apamin (N = 7); 2.70 ± 0.37% per 3 min in the absence of apamin (N = 11)), or on BRL 34915, nicorandil and potassium induced responses.

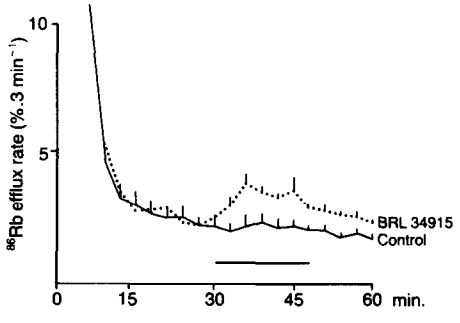

Fig. 2. A typical mean efflux curve from rabbit isolated mesenteric artery (—) and the effect of BRL 34915 on such a curve (. . . .). BRL 34915 (5 μM), when present, is indicated by the horizontal bar. Efflux points are the mean with standard error of 6 experiments.

Cyclic GMP accumulation

Both sodium nitroprusside (100 and 1000 μM) and acetylcholine (100 μM) significantly increased levels of cyclic GMP in rabbit isolated mesenteric artery (Table 2). Whereas BRL 34915 (10 μM) had no effect on basal cyclic GMP levels, nicorandil (100 μM) caused a small, but significant, increase in cyclic GMP concentration.

Cyclic AMP accumulation

Basal levels of cyclic AMP in rat forebrain slices were not affected by BRL 34915 at 100 μM (basal 2.51 ± 0.09 pmoles cyclic AMP/tube; BRL 34915 2.68 ± 0.12 pmoles cyclic AMP/tube; N = 3). Noradrenaline (100 μM) produced a 4-fold increase in cyclic AMP levels which was also not significantly affected by BRL 34915 at 100 μM (noradrenaline 9.4 ± 0.9 pmoles cyclic AMP/tube; noradrenaline +

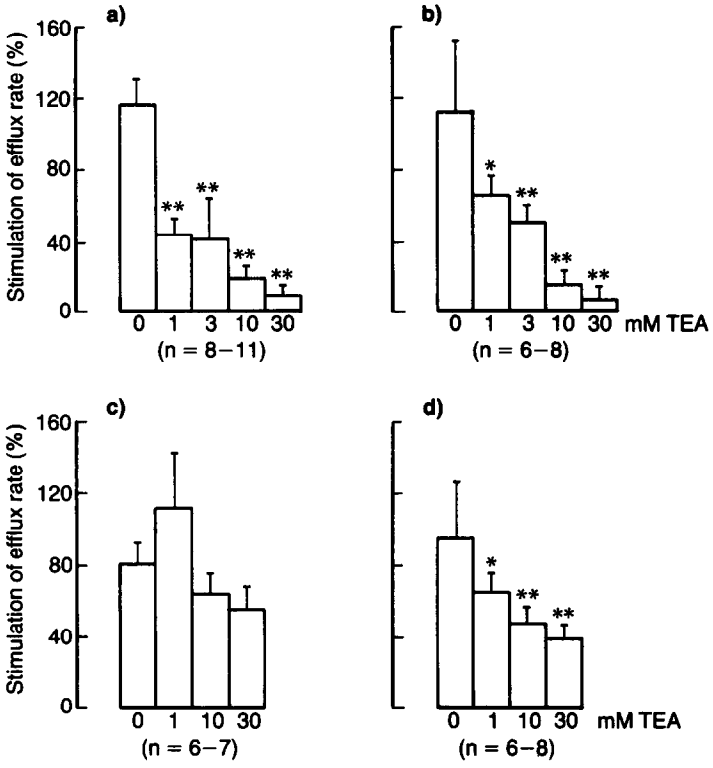

Fig. 3. Effect of tetraethylammonium (TEA) on 86-rubidium efflux from rabbit isolated mesenteric artery stimulated by (a) BRL 34915 (10 μM), (b) nicorandil (100 μM), (c) noradrenaline (30 μM) and (d) potassium (30 mM). Stimulation of efflux rate at the indicated TEA concentration is calculated and presented as described in the legend to Fig. 1. *P < 0.05, **P < 0.01 versus control stimulation in the absence of TEA.

Table 1. Comparison of the effect of BRL 34915 on rubidium efflux rate in different arterial preparations of the rabbit

Tissue	Basal rate (% per 3 min)	Max. stimulation (%)	BRL 34915 concentration (μM)	(N)
Pulmonary artery	1.92 ± 0.23	106 ± 18	10	7
Ear artery	2.11 ± 0.19	50 ± 16	1	6
Brachial artery	1.52 ± 0.10	93 ± 27	10	6
Abdominal aorta	1.78 ± 0.18	96 ± 16	3	5

Values are mean ± SEM.
N = number of preparations.

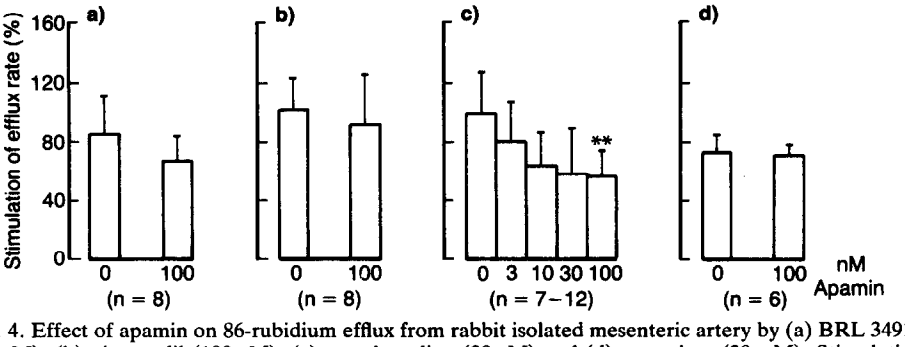

Fig. 4. Effect of apamin on 86-rubidium efflux from rabbit isolated mesenteric artery by (a) BRL 34915 (10 μM), (b) nicorandil (100 μM), (c) noradrenaline (30 μM) and (d) potassium (30 mM). Stimulation of efflux rate at the indicated concentration is calculated and presented as described in the legend to Fig. 1. **P < 0.01 versus control stimulation in the absence of apamin.

Table 2. The effects of BRL 34915, nicorandil and standard compounds on cyclic GMP levels in rabbit isolated mesenteric artery

Treatment (μM)	pmol cyclic GMP/mg protein	(N)	P<
Control	19.6 ± 4.3	26	—
Sodium nitroprusside (100)	158.4 ± 31.6	7	0.01
Sodium nitroprusside (1000)	2195.2 ± 423.9	12	0.01
Acetylcholine (100)	98.1 ± 34.0	8	0.01
BRL 34915 (10)	15.6 ± 3.0	11	NS
Nicorandil (100)	34.5 ± 9.4	12	0.01

Values are means ± SEM from the indicated number (N) of experiments.
P values—Student's *t*-test.

BRL 34915 6.6 ± 0.4 pmoles cyclic AMP/tube; N = 3).

Cyclic AMP and cyclic GMP PDE activity

BRL 34915 was compared with a number of standard compounds for its ability to inhibit rat heart cyclic AMP and cyclic GMP PDE activity. Papaverine was the most potent drug tested with an IC_{50} value of *ca.* 4 μM for both cyclic AMP and cyclic GMP PDE activity. The dihydropyridine nifedipine showed some selectivity for inhibiting cyclic GMP PDE activity (IC_{50} values of 30 μM for cyclic GMP PDE but >100 μM for cyclic AMP PDE) but had overall weaker activity than papaverine. BRL 34915, however, had negligible effects at 100 μM against both enzymes.

Inositol phosphate turnover

Both lithium (5 mM) and BRL 34915 (10 μM) caused a 16 ± 4% (N = 4) increase in the basal inositiol phosphate accumulation in rat cortical slices. In the presence of lithium, noradrenaline (100 μM), 5-HT (1 mM) and carbachol (1 mM) stimulated inositol phosphate accumulation by 90.4 ± 9.7% (N = 19), 54.9 ± 7.3% (N = 15) and 116.0 ± 19.0% (N = 4), respectively. Noradrenaline did not stimulate inositol phosphate accumulation in the presence of BRL 34915, but the absence of lithium.

Prazosin caused a significant inhibition of noradrenaline-induced inositol phosphate accumulation in the presence of lithium, with a K_i value for prazosin calculated at 0.4 nM. BRL 34915, however, had no effect on noradrenaline-induced accumulation.

[^{3}H]-*Nitrendipine binding*

The dihydropyridines tested were found to have high affinity for the [^{3}H]nitrendipine binding site on rat heart membranes (nifedipine 2.6 ± 1.3 nM; nitrendipine 2.3 ± 1.3 nM; felodipin 2.3 ± 1.2 nM; N = 3) whilst BRL 34915 produced less than 50% inhibition at 100 μM.

Neurotransmitter binding sites

A variety of neurotransmitter binding sites were labelled on rat cerebral membranes. In all cases BRL 34915 was compared with known compounds for its ability to compete for binding to these sites. BRL 34915, however, had negligible affinity (IC_{50} of >100 μM) for 5-HT_1, 5-HT_2, α_1- and α_2-adrenoceptors, β-adrenoceptors and dopamine binding sites.

[^{3}H]-*BRL* 34915 *binding sites*

Extensive manipulations of assay conditions and techniques failed to reveal any specific binding site for BRL 34915 in any tissue *in vitro*. Using charcoal and centrifugation as separation methods, it was possible to demonstrate displaceable binding in a number of tissues. However, inhibition of [^{3}H]-BRL 34915 binding only occurred at BRL 34915 concentrations greater than 1 μM.

Attempts were made to label BRL 34915 binding sites *in vivo* by dosing rats with subcutaneous injections of 20 μCi (*ca.* 0.9 μg/kg) [^{3}H]-BRL 34915. However, there was little or no displacement of [^{3}H]-BRL 34915 from the tissues by a 300-fold greater dose of unlabelled BRL 34915.

DISCUSSION

It has been proposed that the anti-hypertensive activity of BRL 34915 is associated with its ability to relax vascular smooth muscle by opening potassium channels, thereby holding the membrane potential close(r) to the potassium equilibrium potential (this membrane shunt inhibiting the effects of stimulatory agents) [4]. The results of the present experiments add further support to this proposal as they demonstrate that BRL 34915 increases rubidium efflux from a number of vascular tissues through channels that are different from those opened by noradrenaline and potassium. Furthermore, examined against a number of secondary messenger systems or at a variety of receptor binding sites, BRL 34915 only had effects at concentrations greater than 100 μM.

The validity for using 86-rubidium as a marker for potassium has been described previously [21] and in addition Bolton and Clapp [22] have shown that in rabbit arteries there is no qualitative difference between ^{42}K and ^{86}Rb efflux. They also showed a wide variation in the maximum rubidium efflux evoked by both noradrenaline and potassium in rabbit vascular tissues and concluded that potassium channels of different vascular smooth muscles exhibit considerable heterogeneity. However, in our experiments with rabbit mesenteric, pulmonary, ear and brachial arteries, and abdominal aorta, the maximum stimulation of rubidium efflux and the maximum evoked efflux caused by BRL 34915 both lay within

a smaller range than reported for noradrenaline or potassium-induced efflux in these tissues [22]. This suggests a degree of homogeneity in the potassium channels activated by BRL 34915 between different vascular tissues.

TEA, which has been shown to block potassium channels in vascular smooth muscle [23], was capable of completely inhibiting the increase in rubidium efflux from rabbit isolated mesenteric artery caused by both BRL 34915 and nicorandil. However, in the concentration range studied, noradrenaline stimulated efflux was not inhibited by TEA and potassium (30 mM) induced efflux was not totally inhibited. These latter results, which are in agreement with those of Bolton and Clapp [22] in rabbit aorta, again show a difference between the potassium channels opened by BRL 34915 (and nicorandil), and those opened by noradrenaline and potassium.

It has been shown previously [7] that the rubidium efflux stimulated by BRL 34915 and nicorandil in the guinea-pig taenia caeci is not sensitive to the bee venom toxin, apamin, although noradrenaline induced efflux was totally inhibited at 100 nM apamin. The present results show that in rabbit vascular tissue, the potassium channel opened by BRL 34915 and nicorandil is also insensitive to apamin whilst the efflux stimulated by noradrenaline is, in part, blocked by apamin.

The stimulation by nicorandil of 86-rubidium efflux from rabbit isolated mesenteric artery appears to occur through a channel with characteristics very similar to those of the channel opened by BRL 34915, with the exception that maximum stimulation of efflux requires a ten-fold higher concentration of nicorandil. This difference is in agreement with previous rubidium efflux studies in rat blood vessels [6]. The results obtained with nicorandil on cyclic GMP accumulation confirm those of Holzmann [14] and agree with previous reports [6, 13] which suggest that the relaxant effects of nicorandil are not due only to the compound's ability to hyperpolarise the smooth muscle membrane.

These results and the observation that the inhibtion by BRL 34915 of spontaneous activity in rat portal veins occurs at concentrations of BRL 34915 that do not cause hyperpolarisation of the membrane [4], prompted us to investigate whether BRL 34915 was having an effect on other second messenger systems. BRL 34915 has been shown to antagonise contractions of rabbit isolated mesenteric artery induced by electrical stimulation and, to a lesser extent, those produced by exogenous noradrenaline [24]. BRL 34915 did not, however, appear to have effects on α-adrenoceptors or α-adrenoceptor-mediated cyclic AMP accumulation or phosphatidyl inositol turnover in tissue slices. Furthermore, inhibition of rat heart cyclic AMP or cyclic GMP PDE activity and effects on a range of other receptors was only demonstrated at concentrations greater than 100 μM,

Preliminary results from our laboratories [27] showed some differences between the pharmacological activities of nifedipine and BRL 34915 in rat portal vein, but also demonstrated that both compounds antagonised the spontaneous contractions of this tissue, which are highly dependent on extracellular calcium. The slow inward calcium channel involved in spontaneous contractions, was examined using [^{3}H]-nitrendipine binding (for review see ref. 28). BRL 34915, however, showed no competition at the [^{3}H]-nitrendipine binding site and was also unable to modify binding by the allosteric mechanism described for diltiazem [29] (data not shown). Furthermore, it has been shown that BRL 34915 has no effect on 45-calcium uptake [30]. It is therefore unlikely that BRL 34915 has any effect on the slow inward calcium channel.

The high potency of BRL 34915 as an anti-hypertensive agent [3], together with stereospecificity of the action exhibited by the (+) and (−) enantiomers, suggests that a specific recognition site exists for the compound [27]. However, our attempts to label this site with [^{3}H]-BRL 34915 were unsuccessful. Although many tissues were used, it is possible that the precise site of action of BRL 34915 may have been omitted. Alternatively, if the sites are to be found exclusively in blood vessel walls, particularly arterioles, their number may be too small to be identified by a ^{3}H-ligand. It is also possible that the kinetics of the drug/receptor interaction are so rapid that all of the techniques available fail to separate bound and free radioligand without totally disrupting the drug/receptor equilibrium.

In summary, we have shown that the potassium channels activated by BRL 34915 are different from those opened by noradrenaline and potassium, and are located in a number of vascular tissues. Nicorandil has a profile similar to BRL 34915 in rubidium efflux studies, but has additional effects (not shared by BRL 34915) which probably also contribute to its relaxant properties. We have been unable to detect any significant effect of BRL 34915 in a variety of other second messenger and receptor systems. Thus the results of the present studies provide further evidence that BRL 34915 relaxes vascular smooth muscle by a novel, and specific, mechanism involving enhancement of potassium efflux through a TEA sensitive channel.

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