The effects of BRL 34915 and nicorandil on electrical and mechanical activity and on ^{86}Rb efflux in rat blood vessels

Sheila W. Weir[1] & A.H. Weston[2]

Smooth Muscle Research Group, Department of Pharmacology, Materia Medica and Therapeutics, Medical School, University of Manchester, Manchester M13 9PT

1   The effects of the antihypertensive agent BRL 34915 on a variety of responses of the aorta and portal vein of the rat have been compared with those of nicorandil.

2   On portal vein, BRL 34915 ($0.01–50 \times 10^{-6}$ M) and nicorandil ($0.1–500 \times 10^{-6}$ M) abolished spontaneous mechanical activity and reduced mechanical responses to noradrenaline ($0.1–100 \times 10^{-6}$ M) and K$^+$ ($5–20 \times 10^{-3}$ M) but had little inhibitory effect on responses to K$^+$ ($40–80 \times 10^{-3}$ M). The onset of the reduced responses to noradrenaline was delayed by both agents.

3   On portal vein, BRL 34915 ($0.1–50 \times 10^{-6}$ M) and nicorandil ($0.5–500 \times 10^{-6}$ M) abolished spontaneous electrical and mechanical activity, hyperpolarized the smooth muscle cells to a value close to their calculated potassium equilibrium potential and increased the ^{86}Rb efflux rate coefficient.

4   On aorta, BRL 34915 ($0.2–0.8 \times 10^{-6}$ M) and nicorandil ($8–32 \times 10^{-6}$ M) reduced mechanical responses to noradrenaline ($0.001–1 \times 10^{-6}$ M) and K$^+$ ($5–20 \times 10^{-3}$ M) but had little inhibitory effect on responses to K$^+$ ($40–80 \times 10^{-3}$ M).

5   On aorta, BRL 34915 ($0.2–0.8 \times 10^{-6}$ M) increased the ^{86}Rb efflux rate coefficient whereas nicorandil ($8–32 \times 10^{-6}$ M) was without effect.

6   It is concluded that the inhibitory actions of BRL 34915 on both aorta and portal vein result from the opening of membrane potassium channels. The resulting membrane shunt inhibits the effects of excitatory agents. The inhibitory effects of nicorandil result from a combination of the opening of potassium channels together with an additional, undefined action.

Introduction

In the accompanying paper (Hamilton *et al.*, 1986), it has been shown that BRL 34915, a new benzopyran derivative with antihypertensive properties in animals (Buckingham *et al.*, 1986), is capable of hyperpolarizing the cells of rat portal vein and inhibiting responses to excitatory agents. Since these effects were also associated with an increased loss of ^{86}Rb from the tissue it was concluded that the inhibitory action of BRL 34915 was mediated by the opening of potassium channels.

It has also been reported that the anti-anginal agent, nicorandil (Sakai *et al.*, 1983), produces inhibitory effects in vascular smooth muscle by opening potassium channels (Inoue *et al.*, 1983; 1984; Kajiwara *et*

al., 1984). However, the presence of a nitro group within the nicorandil molecule has led to the suggestion that some of its inhibitory effects are mediated in an as yet unknown manner, characteristic of other nitro group-containing vasodilators (Inoue *et al.*, 1983; 1984; Kajiwara *et al.*, 1984).

The objective of the present study was to obtain further evidence about the potassium channel-opening properties of BRL 34915 and nicorandil and to determine whether there were any similarities in the actions of the two drugs.

Methods

All tissues were obtained from male Wistar rats ($250–350$ g) supplied by the Manchester University Animal Unit.

[1] Present address: Department of Pharmaceutical Research, Ciba Geigy Limited, CH-4002, Basle, Switzerland.
[2] Author for correspondence.

Tissue bath studies

Portal veins were mounted for isometric recording of tension changes as previously described (Hamilton *et al.*, 1986). After the initial equilibration period, responses of the veins to noradrenaline were investigated by constructing sequential concentration-effect curves. For potassium chloride (KCl), cumulative concentration-effect curves were constructed using a five minute contact time for each concentration. Mechanical responses were quantified using integrators (Hamilton *et al.*, 1986).

A 3 cm length of thoracic aorta was dissected free from surrounding connective tissue. It was placed in cold MOPS physiological salt solution (MOPS–PSS) and cut into 4 rings. Each ring was approximately 0.5 cm long and was opened into a flat sheet by cutting along its longitudinal axis. No attempt was made to remove the vascular endothelium. A thread was attached by means of a small bent pin to each of the longitudinally cut edges.

Tissues were then mounted for isometric tension recording in a 20 ml organ bath containing oxygenated MOPS–PSS maintained at 37°C and pH 7.4. The segments of aorta were suspended under 1 g tension and allowed to equilibrate for 90 min before exposure to a spasmogen. The effects of noradrenaline and potassium were studied by constructing cumulative concentration-effect curves. For noradrenaline, sufficient contact time was allowed for each concentration to develop its maximum tension response and for KCl, a 5 min contact time was employed for each concentration.

Both BRL 34915 and nicorandil were allowed to equilibrate with the tissues for 30 min before agonist responses were re-examined in the continuing presence of either BRL 34915 or nicorandil. Concurrent time-matched control tissues were exposed to the appropriate vehicle.

Electrical recording

The method described by Small & Weston (1980) was used to measure intracellular electrical changes in portal vein. After impalement, approximately 3 min was allowed for the electrical recording to stabilize. After this, the cells were exposed to either BRL 34915 or nicorandil in various concentrations until the maximum effect of a given concentration had been achieved (5–10 min).

^{86}Rb efflux

In these experiments ^{86}Rb was used as a K$^+$-marker (Hamilton *et al.*, 1986). The portal veins were removed from nine rats and each was assigned to one of nine experimental groups: (1–4) nicorandil; (5–8)

BRL 34915; (9) control. Thoracic aortae were also removed from some animals and each was cut into 4 rings and opened longitudinally as already described. The anatomical position of each ring was noted (upper, upper middle, lower middle, lower) before it was assigned using a balanced design, to one of seven experimental groups: (1–3) nicorandil; (4–6) BRL 34915; (7) control.

Tissues were attached to a gassing manifold (Hamilton *et al.*, 1986) and after a 30 min equilibration period, loaded with ^{86}Rb, 1 μCi ml^{-1} for 150 min. The ^{86}Rb was then allowed to efflux from the tissues using 2 min collection periods (Hamilton *et al.*, 1986). After 8 such periods (16 min into the efflux), the PSS to which the tissues were exposed contained the following additions for the next 4 collection periods: portal vein groups 1–4, nicorandil 1×10^{-6}, 5×10^{-6}, 50×10^{-6}, 500×10^{-6} M respectively; groups 5–8, BRL 34915 0.1×10^{-6}, 0.5×10^{-6}, 5×10^{-6}, 50×10^{-6} M respectively; group 9, none; aorta groups 1–3, nicorandil 8×10^{-6}, 16×10^{-6}, 32×10^{-6} M respectively; groups 4–6, BRL 34915, 0.2×10^{-6}, 0.4×10^{-6}, 0.8×10^{-6} M respectively; group 7, none. For the remaining periods, the tubes contained PSS alone.

At the end of the efflux period, 1 ml aliquots of PSS were added to 4 ml Rialuma (Lumac) scintillation mixture and counted for radioactivity. The efflux data were expressed in terms of the rate coefficient (fractional loss of ^{86}Rb from the tissue standardised for a 1 min period, expressed as a percentage).

*Materials and solutions/statistical analysis of results*

The following drugs were used: BRL 34915 (Beecham); (−)-noradrenaline bitartrate (Sigma); nicorandil (May and Baker). ^{86}RbCl was obtained from Amersham. For the tissue bath experiments the stock solutions of BRL 34915 and nicorandil were prepared in 70% v/v ethanol; noradrenaline was dissolved in N/10 HCl. In the ^{86}Rb efflux experiments pure solid nicorandil or BRL 34915 was added to the bulk PSS to avoid the necessity of ethanol controls.

The composition of the physiological salt solution (PSS) used is described in the accompanying paper (Hamilton *et al.*, 1986). When KCl was used as a spasmogen, the stated concentration excludes the KCl (5.9 mM) already present in the PSS.

The significance of differences between means was assessed using a two-tailed unpaired Student's *t* test.

Results

Tissue bath studies

Portal vein Nicorandil ($0.1–1 \times 10^{-6}$ M) abolished
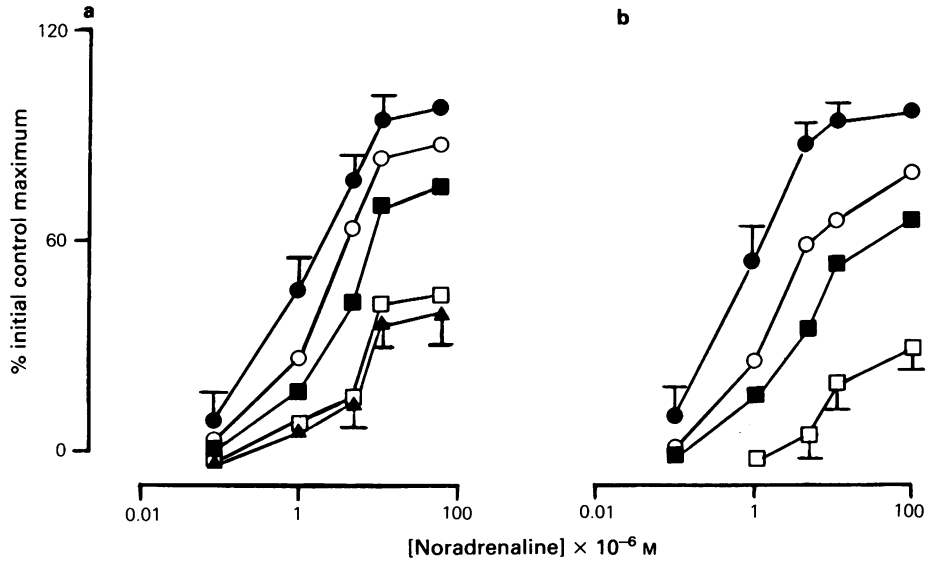
Figure 1 Effect of (a) BRL 34915 and (b) nicorandil on integrated mechanical responses to noradrenaline in rat portal vein. (a) Control responses (●); responses in the presence of BRL 34915, 0.1×10^{-6} M (○); 0.5×10^{-6} M (■); 5×10^{-6} M (□); 50×10^{-6} M (▲). (b) Control responses (●); responses in the presence of nicorandil, 5×10^{-6} M (○); 50×10^{-6} M (■); 500×10^{-6} M (□). Ordinate scale: % of the initial control maximum response. Each point is the mean derived from 6 experiments; vertical lines show sample s.e.mean values.
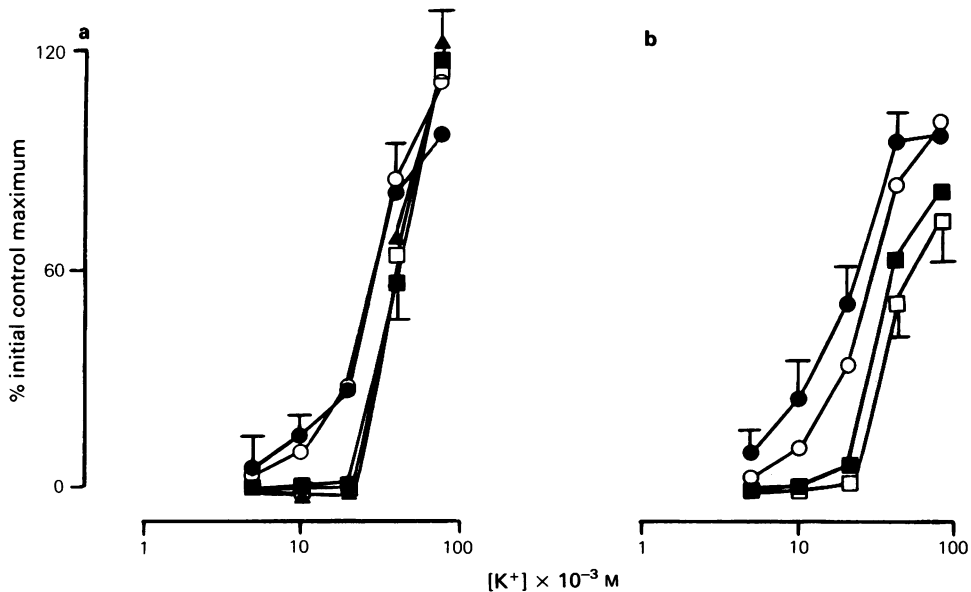
Figure 2 Effect of (a) BRL 34915 and (b) nicorandil on integrated mechanical responses to K^+ in rat portal vein. (a) Control responses (●); responses in the presence of BRL 34915, 0.1×10^{-6} M (○); 0.5×10^{-6} M (■); 5×10^{-6} M (□); 50×10^{-6} M (▲). (b) Control responses (●); responses in the presence of nicorandil, 5×10^{-6} M (○); 50×10^{-6} M (■); 500×10^{-6} M (□). Ordinate scale: % of the initial control maximum response. Each point is the mean derived from 6 experiments; vertical lines show sample s.e.mean values.

spontaneous mechanical activity within 1–2 min. As the concentration was increased (up to 50×10^{-6} M), total inhibition was observed within a few seconds. Pretreatment of tissues with nicorandil ($5–500 \times 10^{-6}$ M) produced a concentration-dependent reduction in responses to noradrenaline (Figure 1). The development of these reduced responses was accompanied by a delay which was similar in magnitude to that described for BRL 34915 in the accompanying paper (Hamilton et al., 1986). Responses to added K^+ were abolished (K^+, $5–20 \times 10^{-3}$ M) or inhibited (K^+, $40–80 \times 10^{-3}$ M) by nicorandil ($5–500 \times 10^{-6}$ M) (Figure 2).

The inhibitory effects of BRL 34915 were similar to those already described by Hamilton et al. (1986). In the present study, increasing the concentration of BRL 34915 to 50×10^{-6} M produced no further inhibitory effects (Figures 1 and 2).

Aorta BRL 34915 ($0.2–0.8 \times 10^{-6}$ M) and nicorandil ($8–32 \times 10^{-6}$ M) each produced a concentration-dependent reduction in responses to noradrenaline (Figure 3). In contrast to the portal vein, neither agent appeared to delay the onset of the reduced responses to noradrenaline. Pretreatment of the aorta with either BRL 34915 or nicorandil also produced a characteristic inhibition of responses to added K^+ similar to that observed in portal vein (Figure 4).

The inhibitory effects of BRL 34915 and of nicorandil on both portal vein and aorta were readily reversible by washing.

Effects on spontaneous electrical and mechanical activity in portal vein

In this series of experiments, the resting membrane potential of portal vein cells was -58.3 ± 0.4 mV (mean $\pm$ s.e.mean, $n = 49$). Both BRL 34915 ($0.1–50 \times 10^{-6}$ M) and nicorandil ($1–500 \times 10^{-6}$ M) abolished the characteristic spontaneous multispike complexes of the portal vein and the associated mechanical activity (see Hamilton et al., 1986). At the lower end of these concentration ranges, the abolition of multispike complexes was not accompanied by a detectable hyperpolarization. However, as the concentration of either agent was increased, a concentration-dependent hyperpolarization with a maximum value of approximately 29 mV was observed. These results are summarized in Table 1.

Effects on ^{86}Rb efflux

At concentrations which abolished spontaneous electrical and mechanical activity without detectable hyperpolarization, neither BRL 34915 nor nicorandil produced significant changes in ^{86}Rb efflux from
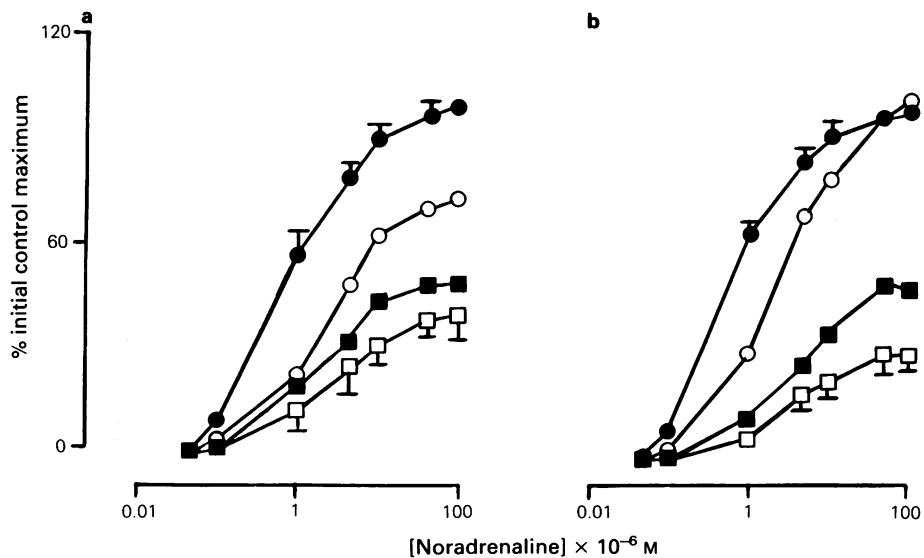
Figure 3 Effect of (a) BRL 34915 and (b) nicorandil on mechanical responses to noradrenaline in rat aorta. (a) Control responses (●); responses in the presence of BRL 34915, 0.2×10^{-6} M (○); 0.4×10^{-6} M (■); 0.8×10^{-6} M (□); (b) Control responses (●); responses in the presence of nicorandil, 8×10^{-6} M (○); 16×10^{-6} M (■); 32×10^{-6} M (□). Ordinate scale: % of the initial control maximum response. Each point is the mean derived from 6 experiments; vertical lines show sample s.e.mean values.
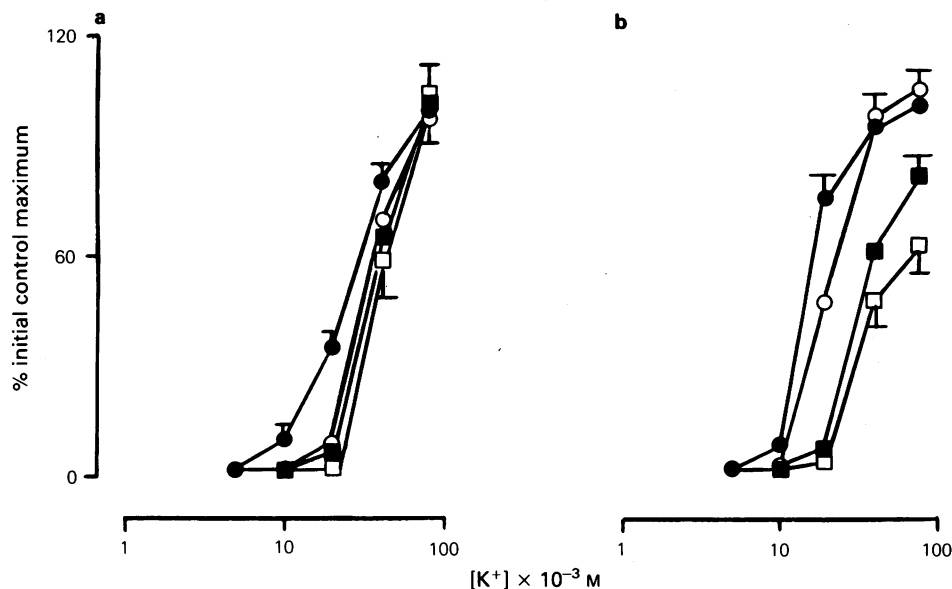
Figure 4   Effect of (a) BRL 34915 and (b) nicorandil on mechanical responses to K $^+$ in rat aorta. (a) Control responses (●); responses in the presence of BRL 34915, 0.2×10^{-6} M (○); 0.4×10^{-6} M (■); 0.8×10^{-6} M (□). (b) Control responses (●); responses in the presence of nicorandil, 8×10^{-6} M (○); 16×10^{-6} M (■); 32×10^{-6} M (□). Ordinate scale: % of the initial control maximum response. Each point is the mean derived from 6 experiments; vertical lines show sample s.e.mean values.

Table 1   Effect of BRL 34915 and nicorandil on resting membrane potential in rat portal vein

| Concentration of BRL 34915 ($\times 10^{-6}$ M)	Hyperpolarization (mV)	Membrane potential (mV)
0.1	0.2 ± 1.0	$- 59.0 \pm 1.4$
0.5	10.0 ± 0.8	$- 66.0 \pm 1.4$
5	27.5 ± 1.1	$- 86.5 \pm 1.5$
50	28.7 ± 1.6	$- 87.2 \pm 2.3$
Concentration of nicorandil ($\times 10^{-6}$ M)		
0.5	0 ± 1.1	$- 58.8 \pm 1.2$
5	11.8 ± 0.9	$- 70.3 \pm 1.9$
50	27.8 ± 1.5	$- 86.7 \pm 2.0$
500	29.5 ± 0.8	$- 89.7 \pm 1.7$

Each value is the mean derived from at least six impalements ± s.e.mean.

portal vein. However, at higher concentrations which were associated with membrane hyperpolarization, both BRL 34915 and nicorandil produced a significant increase in the ^{86}Rb efflux rate coefficient (Figure 5).

In aorta, nicorandil ($8-32 \times 10^{-6}$ M) produced no significant changes in ^{86}Rb efflux, although at the highest concentration used (32×10^{-6} M) there was a trend towards higher values of the ^{86}Rb efflux rate coefficient. At a concentration of 0.2×10^{-6} M, BRL 34915 had no effect on ^{86}Rb efflux. However, at higher concentrations (0.4 and 0.8×10^{-6} M) a small but significant increase in the ^{86}Rb efflux rate coefficient was detected (Figure 6).

Discussion

The results obtained in the present study with BRL 34915 have confirmed and extended those presented by Hamilton et al. (1986). In portal vein, the concentration-dependent hyperpolarization produced by BRL 34915 was paralleled by a similar concentration-dependent increase in ^{86}Rb efflux suggesting a causal relationship between these two events. The inhibition of noradrenaline contractions produced by BRL 34915 also occurred over the same concentration range. Since the maximum hyperpolarization
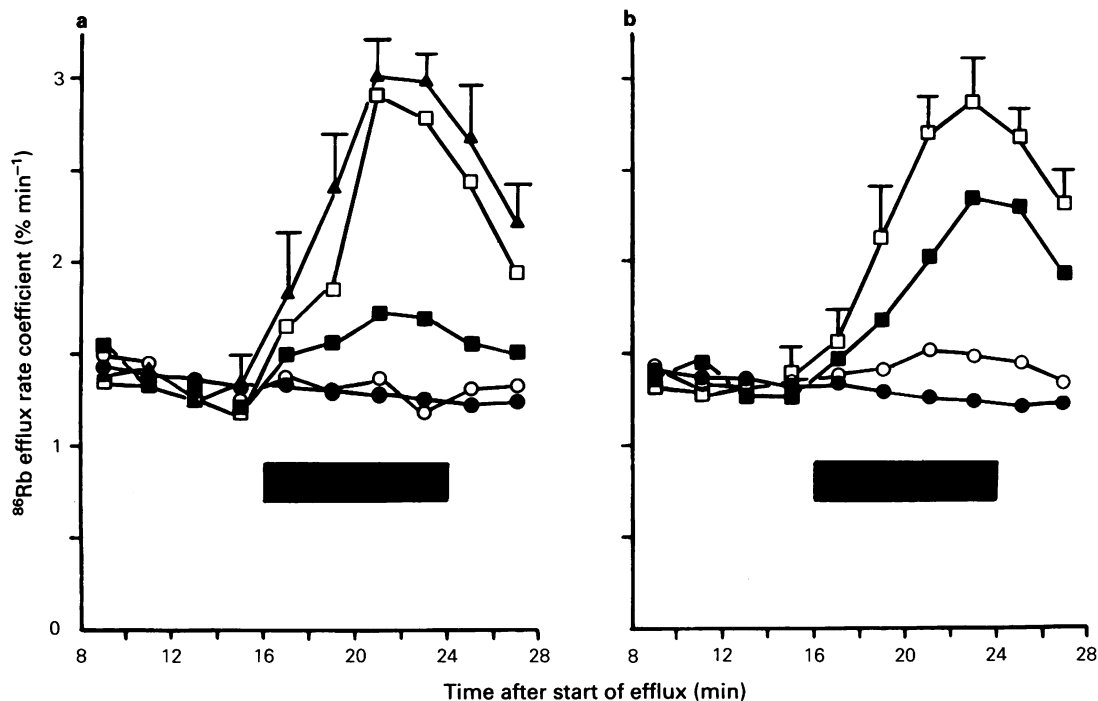
Figure 5 Effect of (a) BRL 34915 and (b) nicorandil on the loss of ^{86}Rb from rat portal vein. (a) Control responses
(●); effect of exposure to BRL 34915, 0.1×10^{-6} M (○); 0.5×10^{-6} M (■); 5×10^{-6} M (□); 50×10^{-6} M (▲), and (b)
control (●); effect of exposure to nicorandil, 5×10^{-6} M (○); 50×10^{-6} M (■); 500×10^{-6} M (□), between the 16th
and 24th min of the effluxing period (■■■). Ordinate scale: ^{86}Rb efflux rate coefficient expressed as a percentage
min^{-1}. Abscissa scale: time (min) after start of effluxing period. Each point is the mean derived from 8 experiments;
vertical lines show sample s.e.mean values.

produced by BRL 34915 was close to the calculated
potassium equilibrium potential for rat portal vein
(see Hamilton et al., 1986), it strongly suggests that the
inhibitory action of BRL 34915 results from the
opening of a population of potassium channels
through which ^{86}Rb can pass. The production of such
a low resistance ionic pathway or membrane shunt
effectively counteracts any inward cation movement
produced by noradrenaline.

No detectable increase in ^{86}Rb efflux was produced
by the low concentrations of BRL 34915 which were
effective inhibitors of spontaneous electrical dischar-
ges in the absence of membrane hyperpolarization.
This could indicate that the expected small increase in
^{86}Rb efflux was obscured by a simultaneous reduction
due to the cessation of spontaneous spike activity.
Such an interaction has been described by Jenkinson &
Morton (1967) in their study of catecholamine action
in guinea-pig taenia caeci.

In portal vein, the inhibitory effects of nicorandil on
spontaneous electrical and mechanical activity and on
noradrenaline contractions and the associated

stimulation of ^{86}Rb efflux were qualitatively similar to
those produced by BRL 34915. However, nicorandil
was approximately ten times less potent than
BRL 34915. A similar finding has also been observed
in guinea-pig taenia caeci (Weir & Weston, 1986).
Both agents produced a delay in the appearance of the
reduced responses to noradrenaline, an action which
was associated with the time taken for noradrenaline
to reverse the hyperpolarizing action of BRL 34915
(Hamilton et al., 1986).

Pretreatment of portal vein with BRL 34915 foll-
owed by exposure to added K$^+$ resulted in the
inhibition of responses only to relatively low concen-
trations of added K$^+$. As already discussed by Hamil-
ton et al. (1986), such an observation is entirely
consistent with the ability of BRL 34915 to open
potassium channels. In the presence of a potassium
channel opener, addition of K$^+$ quickly changes the
cell membrane potential to a value close to the
potassium equilibrium potential. Thus, inhibitory
effects of BRL 34915 against added K$^+$ are only
achieved at potassium equilibrium potentials which
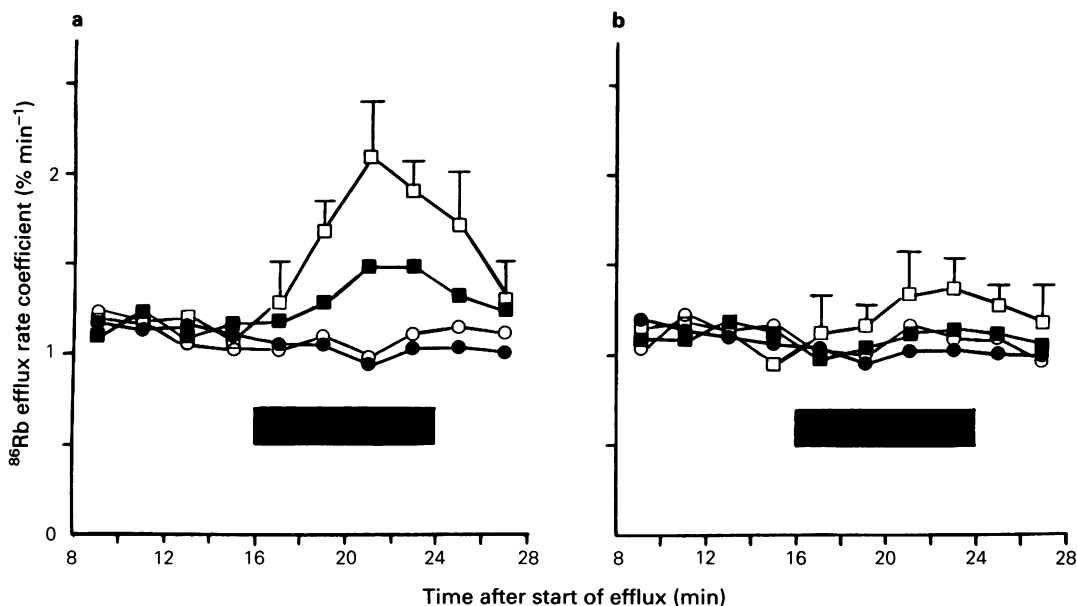
Figure 6 Effect of (a) BRL 34915 and (b) nicorandil on the loss of ^{86}Rb from rat aorta. (a) Control responses (●); effect of exposure to BRL 34915, 0.2 × 10^{-6} M (O); 0.4 × 10^{-6} M (■); 0.8 × 10^{-6} M (□); and (b) control (●); effect of exposure to nicorandil, 8 × 10^{-6} M (O); 16 × 10^{-6} M (■); 32 × 10^{-6} M (□), between the 16th and 24th min of the effluxing period (▬). Ordinate scale: ^{86}Rb efflux rate coefficient expressed as a percentage min^{-1}. Abscissa scale: time (min) after start of effluxing period. Each point is the mean derived from 8 experiments; vertical lines show sample s.e.mean values.

are more negative than the opening potential of the voltage-operated calcium channel. The ability of nicorandil to produce inhibition of responses to high concentrations of added K$^+$ suggests that this drug is capable of inhibiting smooth muscle contraction by a mechanism additional to that of potassium channel opening. Such a suggestion is supported by the greater rightward shift of the noradrenaline concentration-effect curves in this tissue in the presence of nicorandil and by earlier observations in both vascular and bronchial muscles (Inoue et al., 1983; Kajiwara et al., 1984; Allen et al., 1986).

The resting membrane potential of rat aorta is approximately − 50 mV (Jones, 1981) and the calculated potassium equilibrium potential of this tissue is − 87 mV. In these respects the aorta is therefore similar to the portal vein. However, in rat aorta, BRL 34915 was unable to produce an increase in ^{86}Rb efflux comparable to that observed in portal vein. It is possible that the potassium channels in rat aorta do not easily allow the passage of ^{86}Rb but if this were the case, they would be different from those in other smooth muscles (Imaizumi & Watanabe, 1981; Bolton & Clapp, 1984; Allen et al., 1986; Hamilton et al.,

1986; present study). This anomaly cannot yet be satisfactorily explained.

In contrast to BRL 34915, nicorandil produced no significant increase in ^{86}Rb efflux in rat aorta in spite of equivalent or even greater inhibition of responses to both K$^+$ and noradrenaline. These experiments were performed in parallel with those in which a BRL 34915-induced increase was detected. Thus in rat aorta, the inhibitory effects of nicorandil may be primarily associated with mechanisms other than potassium channel opening (see Inoue et al., 1983; Kajiwara et al., 1984; Allen et al., 1986).

The results of the present experiments suggest that the inhibitory effects of BRL 34915 result from the opening of potassium channels. Those of nicorandil seem to result from a potassium channel opening and/ or an unidentified mechanism which may be due to the presence of a NO$_2$ moiety within the nicorandil structure (Maruyama et al., 1982). The nature of the potassium channel affected by these two agents has not yet been determined. However, it appears to be permeable to ^{86}Rb and to remain open at potentials more negative than the resting membrane potential. Further experiments to clarify the nature of the

vascular potassium channel involved are currently in progress. However, studies in guinea-pig taenia caeci have shown that the potassium channel opened by both BRL 34915 and by nicorandil is apamin-insensitive (Weir & Weston, 1986).

References

ALLEN, S.L., FOSTER, R.W., MORGAN, G.P. & SMALL, R.C. (1986). The relaxant action of nicorandil in guinea-pig isolated trachealis. *Br. J. Pharmac.*, (in press).

BOLTON, T.B. & CLAPP, L.H. (1984). The diverse effects of noradrenaline and other stimulants on ^{86}Rb and ^{42}K efflux in rabbit and guinea-pig arterial muscle. *J. Physiol.*, **355**, 43–63.

BUCKINGHAM, R.E., CLAPHAM, J.C., HAMILTON, T.C., LONGMAN, S.D., NORTON, J. & POYSER, R.H. (1986). BRL 34915, a novel anti-hypertensive agent; comparison of effects on blood pressure and other haemodynamic parameters with those of nifedipine in animal models. *J. cardiovasc. Pharmac.*, (in press).

HAMILTON, T.C., WEIR, S.W. & WESTON, A.H. (1986). Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. *Br. J. Pharmac.*, **88**, 103–111

IMAIZUMI, Y. & WATANABE, M. (1981). The effect of tetraethylammonium chloride on potassium permeability in the smooth muscle cell membrane of canine trachea. *J. Physiol.*, **316**, 33–46.

INOUE, T., ITO, Y. & TAKEDA, K. (1983). The effects of 2-nicotinamidoethyl nitrate on smooth muscle cells of the dog mesenteric artery and trachea. *Br. J. Pharmac.*, **80**, 459–470.

INOUE, T., KANMURA, Y., FUJISAWA, T., ITOH, T. & KURIYAMA, H. (1984). Effects of 2-nicotinamidoethylnitrate (Nicorandil; SG-75) and its derivatives on smooth muscle cells of the canine mesenteric artery. *J. Pharmac. exp. Ther.*, **229**, 793–802.

JENKINSON, D.H. & MORTON, I.K.M. (1967). Adrenergic blocking drugs as tools in the study of the actions of catecholamines on the smooth muscle membrane. *Ann. New York Acad. Sci.*, **139**, 762–771.

JONES, A.W. (1981). Vascular smooth muscle and alterations during hypertension. In *Smooth Muscle: An Assessment of Current Knowledge*. ed. Bülbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 397–430. London: Arnold.

KAJIWARA, M., DROOGMANS, G. & CASTEELS, R. (1984). Effects of 2-nicotinamidoethylnitrate (nicorandil) on excitation contraction coupling in the smooth muscle cells of rabbit ear artery. *J. Pharmac. exp. Ther.*, **230**, 462–468.

MARUYAMA, M., SATOH, K. & TAIRA, N. (1982). Effects of nicorandil and its congeners on musculature and vasculature of the dog trachea in situ. *Arch. int. Pharmacodyn.*, **258**, 260–266.

SAKAI, K., NAKANO, H., NAGANO, H. & UCHIDA, Y. (1983). Nicorandil. In *New Drugs Annual Cardiovascular Drugs*. ed. Scriabine, A. pp. 227–242. New York: Raven Press.

SMALL, R.C. & WESTON, A.H. (1980). Simultaneous long-term recording of the mechanical and intracellular electrical activity of smooth muscles. *J. pharmac. Meth.*, **3**, 33–38.

WEIR, S.W. & WESTON, A.H. (1986). Effect of apamin on responses to BRL 34915, nicorandil and other relaxants in the guinea-pig taenia caeci. *Br. J. Pharmac.*, **88**, 113–120