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Cardiovascular effects of apamin and BRL 34915 in rats and rabbits

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1 The cardiovascular effects of apamin, a selective blocker of certain calcium-activated potassium channels, and BRL 34915, a vasodilator thought to act by opening of potassium channels, have been investigated *in vivo* in rats and rabbits.

2 In anaesthetized normotensive rats, apamin (0.05 and 0.15 mg kg^{-1}, i.v.) potentiated angiotensin II pressor responses but did not modify baseline blood pressure or heart rate.

3 Apamin (0.15 mg kg^{-1}, i.v.) was without cardiovascular effects in rabbits.

4 BRL 34915 (0.1 and 0.3 mg kg^{-1}, i.v.) lowered blood pressure in rats dose-dependently and caused reflex tachycardia. The heart rate increase was abolished by prior administration of the β-adrenoceptor blocker bopindolol (0.1 mg kg^{-1}, i.v.).

5 In anaesthetized rabbits, regional blood flow measurements (with radioactive tracer microspheres) showed that BRL 34915 (3 to 30 μg kg^{-1}, i.v.) caused marked vasodilatation in the stomach, with increases in flow also to the heart and small intestine. Brain blood flow also tended to increase. Blood flow to the kidneys was reduced by BRL 34915, whereas flow to skeletal muscle was unchanged.

6 Apamin pretreatment did not modify the blood pressure lowering activity of BRL 34915 in rats. The site at which BRL 34915 acts to cause vasodilatation *in vivo* thus appears to be apamin-insensitive.

Introduction

BRL 34915 is a recently described vasodilator drug, thought to act by opening of smooth muscle membrane potassium (K^+) channels (Hamilton *et al.*, 1986). Stimulation of K^+ efflux from the cell would shift the membrane potential towards more negative values. Hence the opening of voltage-sensitive calcium channels would be opposed and the firing of spontaneously active cells would be inhibited, by moving further away from the threshold potential for activation. In vascular smooth muscle this would be manifested as a reduction in tone (Hamilton *et al.*, 1986) or *in vivo* as a reduction in blood pressure (Buckingham *et al.*, 1986a, b). Two other vasodilator drugs, nicorandil and pinacidil, are now also known to act, at least in part, by a similar mechanism to BRL 34915 (Weir & Weston 1986a; Bray *et al.*, 1987; Cook *et al.*, 1987b).

The mechanism by which BRL 34915 and these other drugs cause activation of K^+ channels, indeed whether this is a direct action or a secondary consequence of some other action, is not yet understood. Recent evidence suggests that the stimulation of ^{86}Rb efflux from pre-incubated blood vessels by BRL 34915 is not mediated through changes in cyclic GMP or cyclic AMP (Southerton *et al.*, 1986; Quast, 1987). Furthermore, the type or types of K^+ channel activated by BRL 34915 are not yet known.

Apamin is a neuropeptide from bee venom which potently blocks a sub-class of calcium-activated potassium channels, most probably those having a low unitary conductance (Jenkinson *et al.*, 1983; Romey *et al.*, 1984; Capiod & Ogden, 1985; Blatz & Magleby, 1986). The inability of apamin to modify BRL 34915-mediated effects in the guinea-pig taenia coli and trachea has recently been described (Weir & Weston, 1986b; Allen *et al.*, 1986). However, no-one has yet studied this interaction at a vascular level. Little is known about the effects of apamin on the cardiovascular system. *In vitro*, apamin was found not to modify contractility of the guinea-pig pulmonary artery or portal vein (Den Hertog *et al.*, 1984) or rabbit aorta (Cook *et al.*, 1987a), in contrast to its prominent effects on certain visceral smooth muscles (Den Hertog *et al.*, 1984; Gater *et al.*, 1985). In the whole animal, apamin (40 μg kg^{-1}) caused an attenuation of α-adrenoceptor-mediated hyperkalaemic responses in both guinea-pigs and rabbits (Coats, 1983; 1985).

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There was also some evidence that the α-adrenoceptor-mediated pressor responses in these animals were potentiated following apamin administration.

In the present study the cardiovascular effects of apamin and BRL 34915 have been investigated *in vivo* in both rats and rabbits. The use of radioactive microspheres to study specific changes in regional blood flows, allows effects on a particular vascular bed to be monitored, which may not be evident from studies of overall systemic haemodynamics. Our results suggest that apamin-sensitive potassium channels may indeed be involved in the regulation of vascular tone in the rat, but not in the rabbit. The effects of BRL 34915 on regional and systemic haemodynamics are also described and evidence is presented that these vascular effects of BRL 34915 are not mediated through apamin-sensitive K^+ channels.

Some of these results were presented at the December 1986 meeting of the British Pharmacological Society (Cook & Hof, 1987).

Methods

Blood pressure studies in rats

Male Wistar rats weighing 300–400 g were anaesthetized with thiobutabarbitone (140 mg kg^{-1}, i.p.) and the trachea cannulated to allow free airways. The rats were then allowed to breathe normal room air. The right femoral artery and vein were cannulated for the measurement of blood pressure and the intravenous administration of drugs respectively. A Statham pressure transducer (Model P23 Gb) was used to record the mean arterial pressure and heart rate was derived from the arterial pulse signal. Blood pressure and heart rate were continuously recorded on an Oki 30 microcomputer and the rats were maintained at a temperature of 36°C.

Following a stabilisation period of 1 h, the baseline blood pressure and heart rate values were recorded (Table 1A). Angiotensin II was then injected i.v. in increasing doses until a blood pressure increase of >20 mmHg was achieved. Blood pressure and heart rate were allowed to return to baseline between each angiotensin II dose. The angiotensin II dose-response curve was repeated to ensure consistent baseline values, after which the drug to be investigated was infused i.v. over a 15 min period in a volume of 1 ml. The angiotensin II dose-response curve was then repeated at regular intervals whilst the blood pressure and heart rate were continuously monitored. A graphical plot of the blood pressure increase due to angiotensin II *versus*, on a log scale, the dose of angiotensin II administered was made for each rat. From this plot, the log of the dose-ratio of angiotensin II causing a 20 mmHg increase in blood pressure before and at various times after drug administration could be determined (i.e. a log dose-ratio of 1 indicates that after drug application 10-times the original predrug dose of angiotensin II was required to elicit the same 20 mmHg blood pressure increase). In certain experiments, depressor responses due to the i.v. administration of isoprenaline were performed, using the same protocol as for the above angiotensin II pressor responses. The log of the dose-ratio of isoprenaline causing a 20 mmHg fall in blood pressure before and at various times after apamin administration was determined by the same procedure as for angiotensin II.

Haemodynamic studies in rabbits

Large mongrel rabbits (body weight 3.5–4.5 kg) were anaesthetized by injection into an ear vein of pentobarbitone 25 mg kg^{-1} followed by 50 mg kg^{-1} phenobarbitone 10 to 15 min later as described in detail elsewhere (Hof, 1985). The animals were tracheotomised 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 % (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 anaesthesia was then deepened by a further injection of phenobarbitone 50 mg kg^{-1}. Through a thoracotomy in the left 3rd intercostal space, the left atrium was cannulated for the injection of microspheres. The aortic root was cleaned of connective tissues and a flowprobe (Narco RT 500, 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 (Hof, 1985).

The use of the microsphere method in our laboratory for determining regional systemic blood flows has been described in detail elsewhere (Hof, 1985). Briefly, for each measurement of regional blood flow about 1.2×10^5 microspheres were injected with one of the following labels: ^{125}I, ^{141}Ce, ^{51}Cr, ^{85}Sr or ^{46}Sc (3M Company). 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^{-1}. After preparation, the rabbits were allowed to stabilize for 60 min. Baseline systemic haemodynamic variables at the end of this period are given in Table 1B. For the BRL 34915 experiments, infusions of $3 + 7 + 20$ μg kg^{-} were made, each over 10 min, to provide total cumulative doses of 3, 10 and 30 μg kg^{-1}. Radioactive microspheres were injected before drug administration and

Table 1 Mean baseline values for the systemic haemodynamic variables of rats and rabbits

A *Rats* *Treatment/Group*	n	*Blood pressure* (mmHg)	*Heart rate* (beats min^{-1})
Apamin controls	8	95 ± 3	339 ± 6
Apamin 0.05 mg kg^{-1}	8	100 ± 3	342 ± 11
Apamin 0.15 mg kg^{-1}	8	87 ± 4	328 ± 15
BRL 34915 0.1 mg kg^{-1}	3	85 ± 4	308 ± 16
BRL 34915 0.3 mg kg^{-1}	3	94 ± 2	310 ± 20
Bopindolol 0.1 mg kg^{-1} + BRL 34915 0.3 mg kg^{-1}	4	94 ± 2	361 ± 11
Apamin 0.15 mg kg^{-1} + BRL 34915 0.3 mg kg^{-1}	3	86 ± 3	302 ± 12

B *Rabbits* *Treatment/Group*	n	*Blood pressure* (mmHg)	*Heart rate* (beats min^{-1})	*Cardiac output* (ml min^{-1} kg^{-1})
BRL 34915	6	70 ± 2	288 ± 18	372 ± 31
Apamin	3	72 ± 2	295 ± 5	331 ± 13
Vehicle for apamin	3	80 ± 2	332 ± 8	377 ± 16

Values are mean ± s.e.mean.

at the end of each infusion period, immediately prior to the next dose, and again 30 min after cessation of infusion of the last dose. In the experiments performed to investigate a possible haemodynamic interaction between apamin and angiotensin II, either apamin (150 μg kg^{-1}) or vehicle (0.9% NaCl) was administered at the end of the 60 min stabilization period. Regional blood flows were then assessed 1 h later by microsphere injection. Further microsphere injections were made towards the end of each 5 min angiotensin II infusion period (doses being 0.03, 0.1 and 0.3 μg kg^{-1}, min^{-1} i.v.). These experimental protocols follow closely those used previously to investigate the haemodynamic effects of calcium antagonists in rabbits (Hof, 1985) thus allowing direct comparisons to be made. At the end of the experiments the animals were killed with an overdose of pentobarbitone. The radioactivity accumulated in the various vascular beds 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 & Heymann (1967) with the modifications of the calculations described by Schosser *et al.* (1979). Relative blood flows are expressed as the percentage of the distribution of cardiac output to the tissues as measured directly by the microsphere technique. Absolute blood flows were calculated by multiplying these relative flows (%) by cardiac output and normalizing for organ weight.

Drugs and solvents used

Angiotensin II was obtained from Bachem, isoprenaline HCl from Sigma, pentobarbitone from Siegfried Zofingen, phenobarbitone (Luminal) from Merck, thiobutabarbitone (Inactin) from Byk Gulden. Bopindolol was synthesized at Sandoz Ltd. BRL 34915 was a gift from Beecham Pharmaceuticals. The structures and full chemical names of bopindolol and BRL 34915 are given in Figure 1. We are grateful to Dr P.N. Strong (University College, London) for the supply of apamin. The apamin used in this study has been 'bioassayed' for its ability to inhibit the Ca^{2+}-activated K^+ permeability of guinea-pig hepatocytes (see Cook & Haylett, 1985) and found to have an IC_{50} of around 1 nM.

In previous studies it has been shown that an i.v. dose of 0.04 mg kg^{-1} apamin is sufficient to attenuate greatly the hyperkalaemic response to α-adrenoreceptor agonists in both guinea-pigs and rabbits (Coats, 1983; 1985), which is thought to be due to a blockade of Ca^{2+}-activated K^+ channels (e.g. in the liver). Based

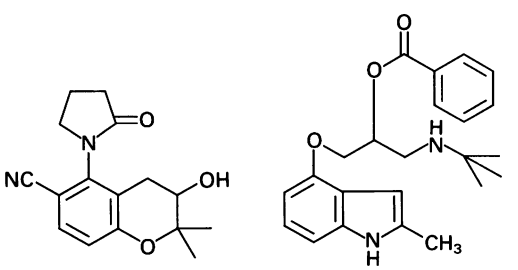

Figure 1 Chemical structures of (left) the K^+ channel opener BRL 34915 ((±)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2H-benzo [b]pyran-3-ol) and (right) the long acting β-adrenoceptor blocker bopindolol (4-[benzoyloxy-3-tert-butylaminopropoxy]-2-methylindole hydrogen malonate). In this study the racemic form of both compounds was used.

on these findings, apamin doses of 0.05 and 0.15 mg kg^{-1} were chosen for the present studies with rats and rabbits.

Apamin, angiotensin II and isoprenaline were dissolved in isotonic saline. For the rat experiments, 1 mg ml^{-1} stock solutions of BRL 34915 and bopindolol were made up in 0.3 ml ethanol and 0.7 ml 5% glucose. Further dilutions were made with 5% glucose as necessary to provide a 1 ml infusion volume per animal. For the rabbit studies, 1 mg of BRL 34915 was dissolved in 0.2 ml ethanol, 0.2 ml polyethylene glycol 400 and 2 ml 5% glucose and infused at an appropriate rate per kg body weight to give the desired dose.

Statistical analyses

Statistical analyses were performed using the RS/1 statistical packages (Bolt, Beranek and Newman Inc. Software Products Corporation). Two-way analysis of variance was used to compare the effects of BRL 34915 in rats in the presence and absence of apamin. Effects of apamin in rats and blood flows in the rabbit experiments were analysed by use of Student's unpaired and paired t tests respectively.

Results

Cardiovascular effects of apamin in rats

The haemodynamic effects of apamin (0.05 and 0.15 mg kg^{-}, i.v.) in rats are shown in Figure 2. A 15 min infusion of apamin had no significant effect on resting blood pressure or heart rate over the following 4 h. Apamin did, however, cause a dose-related increase in the pressor responses caused by angiotensin II injection. This was seen as a leftwards shift of the angiotensin II dose-response curve, represented in Figure 2 as a negative angiotensin II log dose-ratio. This enhancement of angiotensin II responses by apamin reached a peak 2 to 3 h after apamin administration and persisted for longer than 4 h. Control pressor responses to angiotensin II changed little during this period (Figure 2). In 4 of the experiments, isoprenaline depressor responses were established instead of angiotensin II responses. As can be seen in Figure 2, there was a tendency for apamin to attenuate these isoprenaline depressor responses, resulting in a rightwards shift of the isoprenaline log dose-response curve. However this effect of apamin reached significance in only a few cases and showed no obvious dose-dependency.

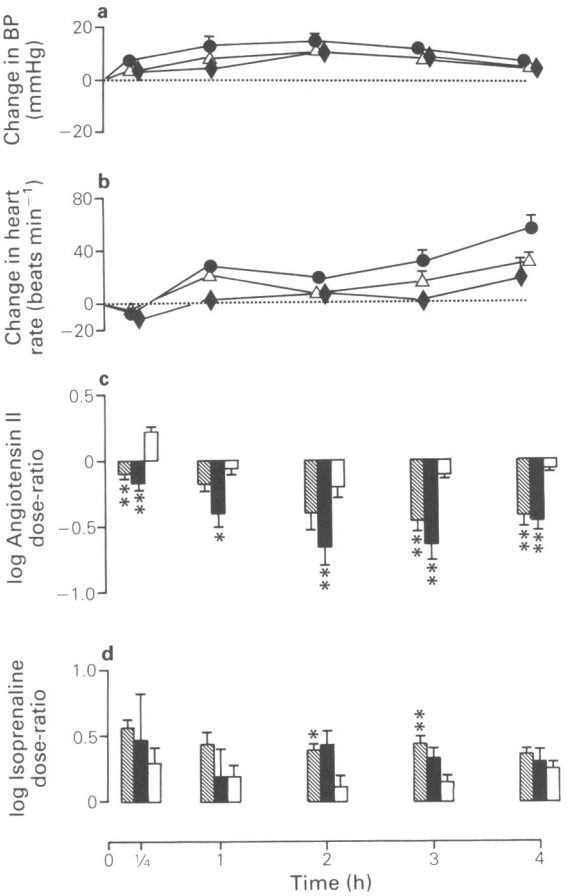

Figure 2 Cardiovascular effects of apamin in rats. Apamin was administered i.v. (15 min infusion) at doses of 0.05 (△, $n = 8$) or 0.15 (●, $n = 8$) mg kg^{-1}. Control animals received the vehicle for apamin (isotonic saline, ◆, $n = 8$). (a and b) Show effects of apamin on blood pressure (BP) and heart rate. (c and d) Show the effects of apamin on pressor responses to angiotensin II ($n = 4$) or depressor responses to isoprenaline ($n = 4$) in these rats. The results are given as the log dose-ratio for angiotensin II required to raise blood pressure by 20 mmHg after apamin (or control) compared to the doses required prior to apamin (or vehicle) administration. For isoprenaline, the log dose-ratio required for a 20 mmHg fall in blood pressure was determined. Hatched columns represent data from animals receiving 0.05 mg kg^{-1} apamin, solid columns 0.15 mg kg^{-1} apamin and open columns controls (vehicle-treated). Vertical lines show the s.e.mean, with a significant difference between apamin and control animals shown as $*P < 0.05$ or $**P < 0.01$.

Cardiovascular effects of BRL 34915 in rats

BRL 34915 (0.1 and 0.3 mg kg^{-1}, i.v.) caused dose-dependent reductions in blood pressure in anaesthetized normotensive rats accompanied by large increases in heart rate (Figure 3). At each dose, the reduction in blood pressure was maximal at the end of the infusion (15 min) and then returned within 1 h to a

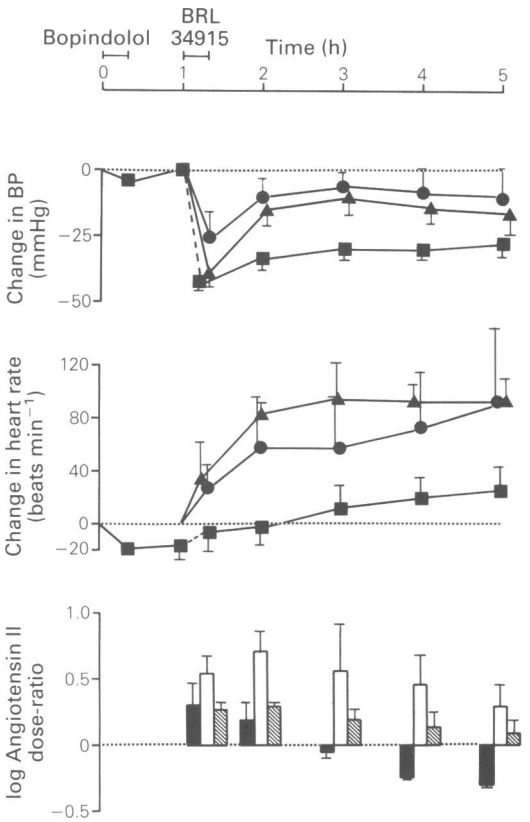

Figure 3 Cardiovascular effects of BRL 34915 in rats. BRL 34915 was administered i.v. to rats over 15 min, as shown by the horizontal bar, at doses of 0.1 (● and solid columns, $n = 3$) or 0.3 (▲ and open columns, $n = 3$) mg kg^{-1}. Changes from baseline of blood pressure, heart rate and effects of BRL 34915 upon angiotensin II pressor responses (see methods for explanation) were then monitored over the following 4 h. In another group of rats, bopindolol (0.1 mg kg^{-1}; ■ and hatched columns) was first administered i.v., followed 1 h later by BRL 34915 (0.3 mg kg^{-1}). Vertical lines represent s.e.mean.

plateau some 10 to 20 mmHg below baseline, at which it remained for the next 3 h. The tachycardia developed more slowly than the fall in blood pressure, a maximal effect being seen 2–4 h after drug application. The long-acting β-adrenoceptor blocker bopindolol (0.1 mg kg^{-1}, i.v.; Berthold *et al.*, 1981; Aellig, 1986) caused a small reduction in heart rate in anaesthetized rats, with minimal effects on blood pressure (Figure 3). Subsequent administration of BRL 34915 resulted in a reduction in blood pressure comparable to that seen in animals not pretreated with bopindolol. However, in these bopindolol-treated rats, the tachycardia normally seen with BRL 34915 was abolished (Figure 3). The fall in blood pressure due to BRL 34915 in the bopindolol pretreated rats remained relatively constant throughout the duration of the experiment, and showed less evidence of the peak effect followed by a plateau which was characteristic of BRL 34915 when given alone. This difference probably reflects the compensatory nature of the reflex increase in heart rate which, in the animals not pre-treated with bopindolol, caused the blood pressure to return towards the initial baseline level, thus counteracting the drug-induced hypotension. BRL 34915 also caused a small attenuation of angiotensin II pressor responses in these rats (Figure 3).

Effects of BRL 34915 in apamin pretreated rats

Apamin (0.15 mg kg^{-1}) was administered intravenously to rats, followed 1 h later by BRL 34915 (0.3 mg kg^{-1}). In Figure 4 it can be seen that the decrease in blood pressure, increase in heart rate and attenuation of angiotensin II pressor responses due to BRL 34915 were similar to those changes seen in rats not pretreated (cf. Figure 3). Two-way analysis of variance showed no statistically significant difference ($P > 0.05$) between the effects of BRL 34915 on blood pressure or angiotensin II pressor responses in the absence or presence of apamin. The effects on heart rate reached a significance level of $P < 0.05$, but this was probably due to the fact that apamin itself had already increased the heart rate from baseline in these animals, thereby limiting the subsequent increase due to BRL 34915. Hence apamin failed to modify the cardiovascular effects of BRL 34915 in rats.

Haemodynamic effects of BRL 34915 in rabbits

The three doses of BRL 34915 (3, 10 and 30 μg kg^{-1}) induced falls in blood pressure in rabbits of 4, 8 and 20% from baseline. Heart rate merely showed a weak tendency to increase in these open chest animals. Cardiac output increased dose-dependently by 1, 6 and 10%. Thirty minutes after the last BRL 34915 dose, blood pressure had returned to a value 8% lower than baseline and cardiac output had completely returned to the pretreatment value.

Figure 5 shows the distribution of cardiac output to several organs. The fraction of cardiac output reaching the heart was increased dose-dependently by BRL 34915, significantly so after the highest dose. The most marked increase in distribution of blood flow was observed in the stomach, whereas the fractions reaching the brain, skeletal muscle and small intestine were unchanged. The kidneys received a markedly smaller fraction of cardiac output after BRL 34915 administration (Figure 5).

Absolute organ blood flow is simply the product of % cardiac output × cardiac output (ml min^{-1} kg^{-1}). Since cardiac output increased dose-dependently,

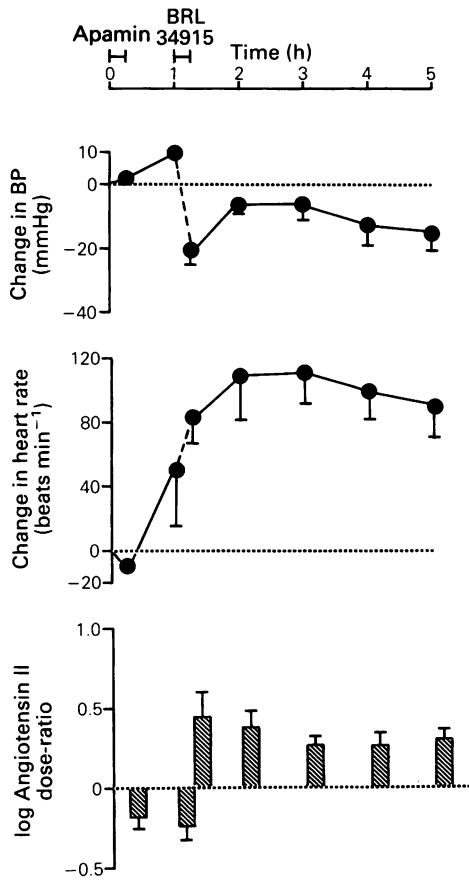

Figure 4 The changes in blood pressure, heart rate and angiotensin II pressor responses induced by BRL 34915 (0.3 mg kg^{-1}) in rats which had been pretreated with the Ca^{2+}-activated K^+ channel blocker apamin (0.15 mg kg^{-1}). BRL 34915 and apamin were infused i.v. during the periods shown by the horizontal bars. Mean results of 3 experiments. Vertical lines represent the s.e.mean.

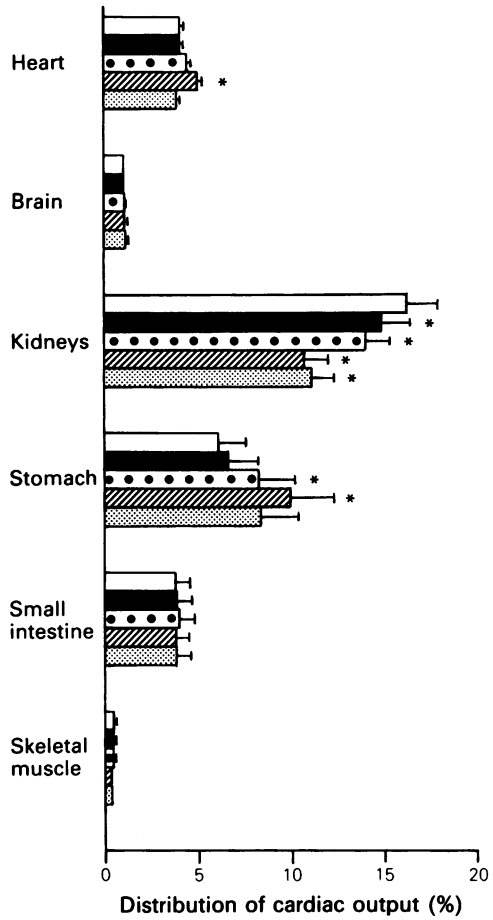

Figure 5 Dose-response relationship for the effects of BRL 34915 on the regional distribution of cardiac output in anaesthetized rabbits. The open columns show the baseline values before treatment. BRL 34915 was infused at 3 different rates, each for 10 min, resulting in the following cumulative doses; 3 (solid columns), 10 (dotted columns) and 30 (hatched columns) μg kg^{-1}. The final measurement was obtained 30 min after the end of the last BRL 34915 infusion (stippled columns). Regional flows are expressed in % of cardiac output as determined by radioactive microsphere injection. Horizontal lines show s.e.mean, $n = 6$. Significant differences from the baseline distribution of cardiac output (open columns) are shown (Student's t test, $^*P < 0.05$).

these results translate into a dose-dependent increase of coronary flow, significant at the highest dose. Brain blood flow showed a tendency (not significant) to increase. Flow to the kidneys remained almost unchanged at the two lower doses, the highest dose, however, inducing a large reduction. A pronounced, dose-dependent increase in flow to the stomach was observed. BRL 34915 elicited a weaker, but also dose-related enhancement of flow in the small intestine. Flow to skeletal muscle was unchanged. As with the effects of BRL 34915 on blood pressure and cardiac output, the flow changes in most vascular beds had subsided 30 min after administration of the last dose (Figure 5).

Lack of haemodynamic effects of apamin in rabbits

Apamin (0.15 mg kg^{-1}, i.v.) had no effect on blood pressure in rabbits compared to placebo (decrease over the experimental time period of 5% in the apamin group and 7% in the group treated with the vehicle of

apamin). The infusions of angiotensin II resulted in pressor responses of 7, 16 and 27% in the apamin group and 4, 20 and 43% in the vehicle-treated group. These small differences were not significant.

The changes in the distribution of cardiac output in the placebo group and the group treated with apamin are shown in Figure 6. The infusion of angiotensin II (3rd to 5th columns) caused comparable changes in

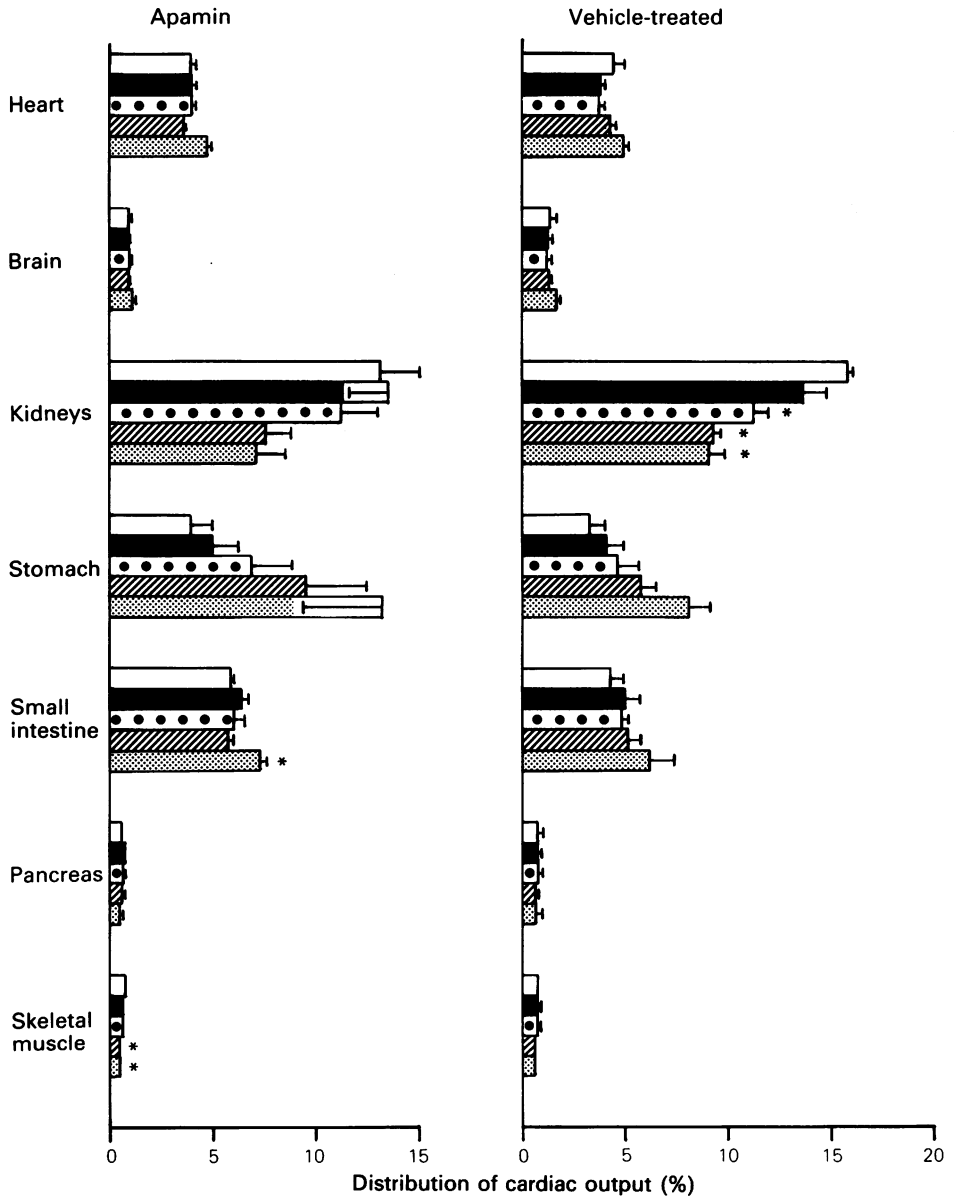

Figure 6 Effects of apamin and angiotensin II on regional % distribution of cardiac output determined with the microsphere method. The open columns show pretreatment values, solid columns show the flow distribution 1 h after the apamin (0.15 mg kg^{-1}, left) or vehicle (0.9% NaCl, right) infusion. Angiotensin II was then infused to each group at doses of 0.03 (dotted columns), 0.1 (hatched columns) and 0.3 (stippled columns) $\mu g\,kg^{-1}\,min^{-1}$. The dose of angiotensin II infused was increased at 5 min intervals, with measurements made towards the end of each infusion period. The horizontal lines show s.e.mean, $n = 3$ per group. Significant differences from the baseline distribution of cardiac output (open columns) are shown (Student's t test, $^*P < 0.05$).

both groups, irrespective of the pretreatment with apamin. The fraction reaching the heart tended to increase, probably due to the increased work of the heart with the pressor effect of angiotensin II. The brain received an unchanged fraction and the fraction reaching the kidneys decreased sharply as an expression of the well known vasoconstrictor effect of angiotensin II in this organ. The fraction of cardiac output flowing to the stomach increased in both groups in parallel to the changes in blood pressure. Angiotensin II induced only trivial changes in the distribution of cardiac output to the small intestine, pancreas and skeletal muscle. However, since cardiac output changed little during angiotensin II administration (2, 9 and 14% increase in the apamin group compared with 1, 4 and 7% in the vehicle-treated animals), and since blood pressure increased, angiotensin II therefore caused vasoconstriction not only in the kidneys but also in the pancreas and skeletal muscle. Apamin did not enhance any of these effects of angotensin II. After performing three such experiments in each group it was obvious that extension to a larger number of animals was not justifiable given the lack of effects of apamin.

Discussion

The results presented in this paper show for the first time that different populations of potassium channels may be involved in the regulation of vascular tone *in vivo*. BRL 34915 caused marked vasodilatation in both rats and rabbits, as is also the case in cats and dogs (Buckingham *et al.*, 1986a). *In vitro* evidence suggests this vasodilatation arises from a direct action on the vascular smooth muscle, where BRL 34915 causes the opening of K^+ channels and membrane hyperpolarization (Hamilton *et al.*, 1986; Weir & Weston, 1986a). The approximate 100 fold difference in potency between the two enantiomers of BRL 34915, both at stimulating ^{86}Rb efflux *in vitro* and for lowering blood pressure *in vivo* (Buckingham *et al.*, 1986b), strengthens the hypothesis that the hypotensive activity of this drug is due to K^+ channel activation.

We now show (Figure 4) that the K^+ channels through which BRL 34915 acts to lower blood pressure *in vivo* are not sensitive to the Ca^{2+}-activated K^+ channel blocker apamin (Jenkinson *et al.*, 1983; Romey *et al.*, 1984; Cook & Haylett, 1985). This agrees with *in vitro* findings in the guinea-pig taenia caeci (Weir & Weston, 1986b), guinea-pig trachealis (Allen *et al.*, 1986) and rabbit aorta (Cook *et al.*, 1987a), where the effects of BRL 34915 are also apamin-insensitive. Whilst these results argue against the possibility that vascular actions of BRL 34915 are mediated through apamin-sensitive K^+ channels, they do not exclude the possibility that BRL 34915 might also be capable of activating such channels as well. However, BRL 34915 is unable to stimulate K^+ efflux from isolated guinea-pig hepatocytes (N. Cook, unpublished observations), which contain such apamin-sensitive channels (Cook & Haylett, 1985), and Weir & Weston (1986b) have found that apamin and BRL 34915 appear to act on distinctly different K^+ channels in the guinea-pig taenia coli.

A characteristic effect of direct-acting vasodilator drugs is to induce tachycardia via a reflex increase in sympathetic drive to the heart. This is true also for BRL 34915 (Figure 3). The heart rate increases due to BRL 34915 were abolished by prior administration of the long-acting β-adrenoceptor blocker bopindolol (Berthold *et al.*, 1981: Aellig, 1986), suggesting this to be a reflex effect rather than a direct action of BRL 34915 on the heart. One consequence of abolishing this tachycardia was a more sustained reduction of blood pressure by BRL 34915 in the bopindolol pretreated rats (Figure 3). Since early clinical trials suggest BRL 34915 also induces tachycardia in man (Van den Burg *et al.*, 1986; Fox *et al.*, 1987), co-administration of a β-blocker with BRL 34915 would seem a likely combination if this drug is to be introduced as an antihypertensive in man.

The results obtained with apamin in the rat (Figure 2), suggest that apamin-sensitive Ca^{2+}-activated K^+ channels may also be involved in blood pressure regulation. Pressor responses to angiotensin II were significantly increased in the presence of apamin, despite the lack of effect of apamin on resting blood pressure. These results are in agreement with those of Coats (1983; 1985), where there was some suggestion that α-adrenoceptor-mediated pressor responses in guinea-pigs and rats were enhanced in the presence of apamin, whilst baseline blood pressure was little affected. Thus it seems that in both rats and guinea-pigs, apamin-sensitive potassium channels may serve a regulatory function, opening during hormonal stimulation (i.e. when intracellular calcium levels rise) to hyperpolarize the membrane, thereby terminating the vasoconstrictor response by closing voltage-sensitive calcium channels. During basal conditions it appears that intracellular calcium levels are insufficient to open these potassium channels, as demonstrated by the lack of effect of apamin on basal blood pressure. In PC12 phaeochromocytoma cells, apamin-sensitive Ca^{2+}-activated K^+ channels have been found to activate over the range 0.1–0.3 μM intracellular free Ca^{2+} (Schmid-Antomarchi *et al.*, 1986). This is precisely the range over which angiotensin II has been shown to modify intracellular Ca^{2+} levels in cultured aortic smooth muscle cells (Smith, 1986; see also Bolton, 1979), thus supporting the conclusion of an effect of apamin on elevated, but not on basal, vascular tone.

A possible interaction between β-adrenoceptor-mediated depressor responses and apamin-sensitive K^+ channels was tested for two reasons. Firstly, phosphorylation by cyclic AMP has been shown to modify the opening of K^+ channels in a number of tissues (see review by Levitan, 1985). Secondly, in guinea-pig liver cells there is a cooperative interaction between agents which raise intracellular levels of Ca^{2+} and those which act through cyclic AMP, with regard to their stimulation of K^+ permeability through apamin-sensitive K^+ channels (Cocks *et al.*, 1984). Such an interaction could imply that part of the vasorelaxant effect of cyclic AMP might be due to membrane hyperpolarization, via the phosphorylation and subsequent opening of K^+ channels (see Johansson & Somlyo, 1980). Although in the present study there was indeed a tendency for apamin to inhibit the isoprenaline-induced depressor responses in rats (Figure 2) this effect showed no obvious dependency on the dose of apamin administered and significance was achieved in only a few instances. Hence the present evidence is not strong enough to support the contention that β-adrenoceptor-evoked responses may be in part be mediated through the opening of apamin-sensitive K^+ channels. This hypothesis clearly merits further investigation.

At a dose of $0.04\,mg\,kg^{-1}$, apamin has been previously shown to attenuate greatly the hyperkalaemic response to α-adrenoceptor agonists in rabbits (Coats, 1985). However, in preliminary experiments with rabbits we found no effect of apamin upon angiotensin II-mediated blood pressure increases, in contrast to the findings in rats (Figure 2). Hence experiments were performed to investigate the possibility that apamin might act only in certain vascular beds in the rabbit, with these effects not being detected in terms of overall systemic haemodynamic changes. Radioactive microspheres were thus used to monitor regional blood flows before and after apamin (or placebo) administration and in the presence of constant angiotensin II infusions. However, these studies failed to detect any clear systemic effects of apamin on peripheral blood vessels (Figure 6). This lack of haemodynamic effects of apamin in rabbits may simply reflect its rapid breakdown in this species. This seems unlikely though in view of its ability to modify plasma K^+ levels in rabbits and its long-lasting effects in rats (Figure 2) and guinea-pigs (Coats, 1983). A second possibility is that the apamin-sensitive channels play little or no role in the regulation of vascular tone in the rabbit, other factors dominating under the experimental conditions employed.

As far as a comparison is possible, the haemodynamic activity of BRL 34915 in rabbits seems similar to that observed in cats (Buckingham *et al.*, 1986a). These similarities extend to changes in blood pressure, heart rate, the small increases in brain and intestinal blood flows and the relative lack of effect of BRL 34915 on blood flow to skeletal muscle (see Buckingham *et al.*, 1986a, who used electromagnetic flow probes to measure carotid, mesenteric, femoral and renal artery blood flow in cats). Only with respect to the renal circulation were marked differences observed, BRL 34915 causing a small increase in cats, whereas the tendency was towards a decrease in renal flow in rabbits (Figure 5). As has been discussed previously, the renal circulation of the rabbit seems to be atypical in its response to vasodilators, calcium antagonists causing a decreased renal flow in rabbits but not in other species (Hof, 1983; 1985).

Standardization of the experimental protocol allows us to compare directly the peripheral vascular effects of the K^+ channel activator BRL 34915 with other vasodilators tested in this model. Calcium antagonists increase coronary and, especially, cerebral blood flow much more than BRL 34915 at doses causing comparable falls in blood pressure (Hof, 1983; 1985). Also, dihydropridines strongly dilate skeletal muscle vessels, while BRL 34915 did not. By contrast, BRL 34915 relatively selectively dilated the stomach vascular bed whereas calcium antagonists have little effect here (Hof, 1983; 1985). The peripheral effects of BRL 34915 were also quite different from the 'non-specific' vasodilator dihydralazine. Whilst the effects on the gastric vascular beds of these two compounds were similar, dihydralazine caused considerably greater vasoldilatation of renal and skeletal muscle blood vessels (Hof & Hof, 1984).

In conclusion, the K^+ channel activator BRL 34915 has a characteristic vasodilator profile, resulting in falls in blood pressure in both rats and rabbits. These effects of BRL 34915 seem not to be mediated through apamin-sensitive K^+ channels. However in rats, as in guinea-pigs (Coats, 1983), a second class of K^+ channels, that are inhibited by apamin, seems also to play a role in the regulation of blood pressure.

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