Br. J. Pharmac. (1984), **82**, 051–060

The calcium antagonists PY 108–068 and verapamil diminish the effects of angiotensin II: sites of interaction in the peripheral circulation of anaesthetized cats

R. P. Hof

Cardiovascular Unit of Preclinical Research Sandoz Ltd, 4002 Basel, Switzerland

1 The sites of interaction between the vasoconstrictor angiotensin II (A II) and the calcium antagonists PY 108–068 (PY) (a dihydropyridine derivative) or verapamil (V) in different peripheral vascular beds were investigated using the microsphere method in chloralose-urethane anaesthetized open-chested cats.

2 A II was infused intravenously into 27 cats at a rate of 0.15 $\mu g\,kg^{-1}\,min^{-1}$. Systemic haemodynamic variables and regional blood flow were measured immediately before and 10 min after the start of the infusion.

3 While the infusion of A II continued, PY (3 $\mu g\,kg^{-1}\,min^{-1}$), V (30 $\mu g\,kg^{-1}\,min^{-1}$) or the vehicle was infused for 10 min into 9 cats each and the effects of this combined infusion were again measured at the end of the 10 min period.

4 A II increased mean arterial blood pressure but decreased peripheral conductance and, to a smaller but still significant degree, cardiac output and peak acceleration of blood in the aorta (an ejection phase parameter of myocardial contractility).

5 The calcium antagonists reversed these effects. Cardiac output and total peripheral conductance were increased even beyond the pre-A II level by PY.

6 A II constricted the vascular beds of the kidney, small intestine, liver and skin. Arterio-venous shunt flow decreased. Vasoconstriction was also found in the stomach, spleen and in different parts of the heart with the exception of the subendocardial layer of the left ventricle, where blood flow increased and conductance remained unchanged. A II did not decrease conductance in different parts of the brain or in skeletal muscle.

7 The vasoconstrictor effects of A II persisted or tended to be increased in most of the vascular beds of placebo treated animals. PY 108–068 and verapamil abolished the vasoconstrictor effects of A II in most of the vascular beds with the exception of the liver, the spleen, the skin and the arterio-venous shunts and caused vasodilatation in the heart. PY also induced vasodilatation in the brain and skeletal muscle, where A II had not induced vasoconstriction.

8 The pattern of attenuation of A II effects was different from the pattern of vasodilatation induced by these and other calcium antagonists in the same cat preparation not treated with a vasoconstrictor. The sites of action of this dihydropyridine derivative (PY) on the peripheral circulation thus, appear to depend not only on the vascular bed but also on the presence of a vasoconstrictor influence at the time of investigation.

Introduction

Angiotensin (A II) is the most potent endogenous vasoconstrictor known. Recent experience with angiotensin converting enzyme inhibitors has shown that A II plays an important role in various, not only the renal, forms of hypertension (Karlberg *et al.*, 1982; Stumpe *et al.*, 1982). In congestive cardiac failure this vasoconstrictor is also important for maintaining an increased peripheral resistance (Morris *et al.*, 1977; Curtiss *et al.*, 1978, Levine & Cohn, 1982).

The action of A II on smooth muscle appears to depend on extracellular calcium (Deth & Van

Breemen, 1974; Freer, 1975; Peach, 1981). There is good evidence that activation of the angiotensin receptor, at least on the rabbit aorta, involves both the activation of a receptor linked channel which allows the influx of extracellular calcium as well as the release of calcium from an intracellular pool (Deth & Van Breemen, 1974; 1977; Healy & Bohr, 1977). We have previously studied this concept in rabbit aortic preparations in which we had depleted the intracellular calcium stores (Hof *et al.*, 1982b). We found, that in this preparation A II allowed extracellular calcium to enter the smooth muscle cells and to initiate contraction. This was not blocked by either the dihydropyridine derivative PY 108–068 (PY) or verapamil (V).

However, in experiments *in vivo* the pressor effect of A II was attenuated by various calcium antagonists (e.g. Ichikawa *et al.*, 1979; Cavero & Lefevre-Borg, 1981; Millar *et al.*, 1981; Vierhapper & Waldhaeusl, 1982). Thus vascular regions must exist, where, in contrast to rabbit aorta, calcium antagonists inhibit the A II induced vasoconstriction. The microsphere method is especially useful in the search for vascular beds, where calcium antagonists might abolish or attenuate A II-induced vasoconstriction in anaesthetized open-chested cats. The experiments were intended to show whether the vasoconstrictor effects of A II were antagonized specifically in those vascular beds where vasodilatation was observed upon administration of typical calcium antagonists or whether the attenuation occurred only in blood vessels constricted strongly by A II.

The dihydropyridine derivative PY 108–068 (PY) appeared to be especially well suited for such an investigation. This compound is a slow channel blocker in electrophysiological experiments on the heart (Scholtysik & Schaad, 1983). Its effects on the peripheral circulation have been thoroughly investigated and compared with nifedipine (Hof *et al.*, 1982a), nicardipine, verapamil and diltiazem (Hof, 1983). Most importantly for this study, we have shown this compound to be highly selective in antagonizing depolarization, but not A II, induced contractions of rabbit aorta (Hof *et al.*, 1982b). PY is, in this respect, dissimilar from verapamil which has been shown to have (unspecific?) affinities for many receptors in experiments *in vitro* (Hof *et al.*, 1982b; Karliner *et al.*, 1982) and in binding studies (Glossmann & Hornung, 1980).

Methods

The preparation of the experimental animals (Hof, 1983) and the use of the microsphere method (Hof *et al.*, 1980; 1981) have been described previously in detail. Both the preparation of the experimental animals and the handling of the microspheres were identical to the methods used by Hof (1983) to facilitate direct comparison of the results recounted here with those obtained previously. The only difference was that larger cats (2.5–5 kg) were used in this series of experiments.

Mongrel cats were anaesthetized (chloralose, 43 mg kg^{-1} and urethane, 430 mg kg^{-1} injected intramuscularly), tracheotomized and ventilated with a Loosco MK2 infant ventilator. Room air was used and a positive end-expiratory pressure was applied as soon as the thorax was opened. The ventilation was adjusted to keep the end-expiratory CO_2 between 4.2 and 4.7 volume % and the arterial blood gases were checked regularly. Catheters were placed in the lower abdominal aorta, the inferior vena cava, the right atrium and, through a thoracotomy, in the left atrium. A flowprobe was placed on the aortic root. The phasic flow signal was integrated to obtain mean aortic flow, and differentiated to obtain dQ/dt, i.e. acceleration of blood in the aorta, which we used as

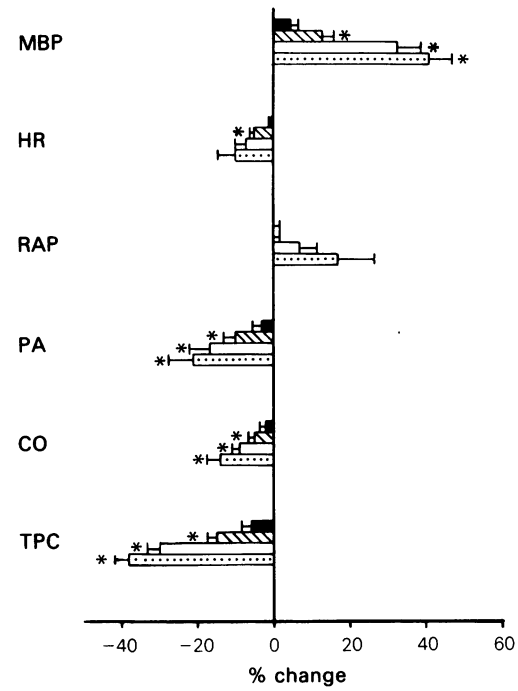

Figure 1 Dose-response relationship for the systemic haemodynamic effect of infusions of angiotensin II (A II), 0.03 (solid columns), 0.07 (hatched columns), 0.15 (open columns) and 0.3 (stippled columns) μg kg^{-1} min^{-1}. The infusion rate of 0.15 μg kg^{-1} min^{-1} was selected for the experiments with regional blood flow measurements. Abbreviations as in Table 1. *$P < 0.05$, significantly different from base line value. The vertical bars show s.e. mean, $n = 8$.

an ejection phase parameter of myocardial function (Hof & Hof, 1981) The electromagnetic flow probe was calibrated *in vivo* by the reference flow method at the time of the last microsphere injection. Total peripheral conductance (TPC) was calculated by dividing cardiac output by mean arterial pressure, neglecting the small right atrial pressure (always smaller than 4 mm Hg, as shown in Table 1). For each determination of regional blood flow we injected about 1.5×10^5 microspheres with one of the following labels: ^{141}Ce, ^{51}Cr, ^{85}Sr or ^{46}Sc. In order to avoid systematic errors due to small differences between different batches of microspheres, spheres with different labels were rotated, so that each label was used for each measuring period. The spheres were injected into the left atrium with 1 ml of 0.9% w/v NaCl solution (saline). This procedure had no effect on blood pressure, heart rate or aortic flow.

At the end of the experiment the animals were killed with an overdose of pentobarbitone and the organs containing radioactivity to be counted were dissected and weighed. Samples of skeletal muscle were obtained from the hindlegs. All other organs mentioned had their radioactivity counted *in toto*. The heart was dissected to obtain samples of the free wall of the left ventricle, which was then divided into 3 layers as previously described in detail (Hof & Hof 1982). The papillary muscles were weighed and counted together with the subendocardial layer. The samples were counted in a Packard γ counter (Model 5921) and the spectra processed on a Hewlett Packard 21 MX minicomputer according to the method of Rudolph & Heymann (1967) with modifications of the calculations described by Schaper *et al.*, (1973).

After the preparative procedures were complete the cats were allowed to stabilize for a minimum period of 90 min. Fresh drug solutions were prepared immediately before the start of each experiment. Angiotensin II (Ciba, Hypertensin) was diluted in 5% glucose to a concentration of 1 $\mu g\,kg^{-1}\,ml^{-1}$. PY

Table 1 Baseline values for the systemic haemodynamic variables, regional blood flow and peripheral conductance, before the first administration of angiotensin II, used to calculate the changes induced by this vasoconstrictor agent

Systemic variables	
HR (beats min^{-1})	212 ± 6.19
MBP (mmHg)	131 ± 3.44
RAP (mmHg)	2.83 ± 0.27
CO (ml $min^{-1}\,kg^{-1}$)	108 ± 4.61
TPC (ml $min^{-1}\,mmHg^{-1}\,kg^{-1}$)	0.84 ± 0.04
Peak acc. (ml s^2)	708 ± 32.8

Organ	*Flow* (ml $min^{-1}\,100\,g^{-1}$)	*Conductance* (ml $min^{-1}\,mmHg^{-1}\,100\,g^{-1}$)
Heart total	139 ± 9.99	1.06 ± 0.07
Epi	170 ± 12.2	1.30 ± 0.09
Mid	188 ± 12.3	1.44 ± 0.09
Endo	190 ± 14.9	1.45 ± 0.11
Brain total	37.0 ± 3.06	0.28 ± 0.02
Cortex	36.6 ± 3.13	0.27 ± 0.02
Cerebellum	38.3 ± 2.97	0.29 ± 0.02
Brainstem	37.7 ± 3.20	0.29 ± 0.02
Kidneys	292 ± 16.6	2.28 ± 0.15
Adrenals	496 ± 35.3	3.79 ± 0.24
Stomach	18.9 ± 2.41	0.14 ± 0.01
Small intestine	45.1 ± 5.48	0.35 ± 0.04
Liver	69.1 ± 6.47	0.53 ± 0.04
Pancreas	45.7 ± 9.69	0.34 ± 0.06
Spleen	114 ± 16.6	0.89 ± 0.13
Muscle	2.73 ± 0.37	0.02 ± 0.00
Skin	3.33 ± 0.28	0.02 ± 0.00
Lungs	120 ± 14.2	0.93 ± 0.11

Microspheres trapped in the lungs represent the sum of the bronchial flow and the much larger flow through arterio-venous shunts (for details see Results).

Abbreviations: epi, mid, endo: subepicardial, middle and subendocardial layer of the left ventricular free wall. HR: heart rate; MBP: mean blood pressure; RAP: right atrial pressure; CO: cardiac output; TPC: total peripheral conductance; peak acc.: peak acceleration of blood in the aorta. These abbreviations are also used in the Figures. The results are expressed as mean ± s.e. mean and $n = 27$ for all measurements.

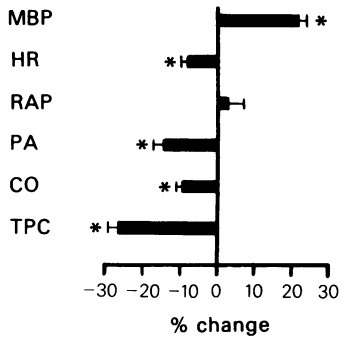

Figure 2 Systemic haemodynamic effects of angiotensin II (A II) 0.15 $\mu g\,kg^{-1}\,min^{-1}$. Abbreviations as in Table 1. * $P<0.05$ significantly different from base-line values before infusion. The verticle bars show s.e. mean, $n=27$.

was dissolved in a mixture of ethanol and polyethyleneglycol 400, 1 ml each per mg of PY. This solution was diluted with 5% glucose to a concentration of 30 $\mu g\,kg^{-1}\,ml^{-1}$. The same amounts of ethanol and polyethyleneglycol were added to the verapamil solution so that only one placebo group was needed.

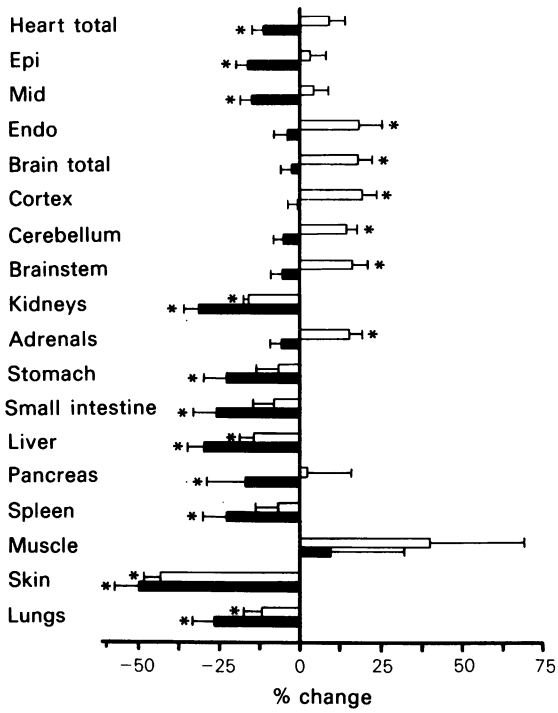

Figure 3 Effects of angiotensin II (A II), 0.15 $\mu g\,kg^{-1}\,min^{-1}$, on regional blood flow (open columns) and conductance (solid columns). * $P<0.05$, significantly different from the preinfusion value. Abbreviations as in Table 1. The vertical bars show s.e. mean, $n=27$.

In preliminary experiments (Figure 1), dose-response curves for A II were performed and a dose increasing blood pressure by 20–30% (steep part of the dose-response curve) was chosen, namely an infusion rate of 0.15 $\mu g\,kg^{-1}\,min^{-1}$. All 27 cats were infused at this rate for 20 min. Haemodynamic measurements were obtained and microspheres were injected just before and 10 min after the start of the infusion. Either PY or verapamil was then infused over a further period of 10 min into 9 of the cats, and 9 further animals were infused with the vehicle for PY and thus served as the control group. Measurements were obtained again at the end of this 10 min period.

In some of the control cats a dose-response curve to A II was carried out at least one h after the end of the previous A II infusion. The results were similar to those obtained in the preliminary experiments and they are also incorporated into Figure 1.

Statistical evaluation

The effects of angiotensin were evaluated by comparing the base line values with the values obtained after 10 min of A II infusion using the *U* test of Wilcoxon. The effects of PY were evaluated by comparing the changes occurring in the control group with the changes induced by the PY or V infusion using the Kruskal-Wallis test. *P* values < 0.05 were considered significant.

Results

The base line values of the systemic haemodynamic variables for all 27 cats used for the microsphere experiments are shown in Table 1. The effects of A II on these variables, measured after 10 min of infusing 0.15 $\mu g\,kg^{-1}\,min^{-1}$ into 27 cats are shown in Figure 2 as changes from these base line values. Mean blood pressure increased whereas heart rate, cardiac output, peak acceleration of blood in the aorta (an injection-phase parameter of myocardial contractility) and total peripheral conductance decreased. All these effects were clearly dose-dependent as demonstrated by the preliminary experiments (Figure 1).

The effects of A II on peripheral blood flow and conductance are shown in Figure 3. A II strongly constricted the blood vessels of the kidney, the small intestine and the liver (arterial blood flow). Microspheres reach the lungs by two routes. When using spheres with a diameter of 15 μm, spheres crossing through arterio-venous shunts outnumbered by far spheres reaching the lungs through the bronchial circulation (Hof *et al.*, 1980). The decrease in 'lung flow' thus indicates mostly a decrease in arterio-venous shunt flow. Skin flow as measured by these

Table 2 Base line values for the systemic haemodynamic variables, regional blood flow and peripheral conductance before the administration of either the vehicle (placebo), PY 108–068 or verapamil

Systemic variables	*Placebo*	*PY 108–068*	*Verapamil*
HR (beats min^{-1})	205 ± 11.1	206 ± 8.32	173 ± 5.77
MBP (mmHg)	161 ± 7.12	153 ± 7.53	163 ± 6.95
RAP (mmHg)	2.68 ± 0.24	3.15 ± 1.09	3.34 ± 0.31
CO ($ml\ min^{-1}\ kg^{-1}$)	104 ± 7.09	85.1 ± 7.52	107 ± 5.13
TPC ($ml\ min^{-1}\ mmHg^{-1}\ kg^{-1}$)	0.66 ± 0.06	0.56 ± 0.05	0.66 ± 0.02
Peak acc. ($ml\ s^2$)	558 ± 34.7	564 ± 42.6	689 ± 38.3
		Blood flow ($ml\ mm^{-1}\ 100g^{-1}$)	
Organ	*Placebo*	*PY 108–068*	*Verapamil*
Heart total	168 ± 32.8	159 ± 27.4	132 ± 14.5
Epi	203 ± 35.4	182 ± 34.4	148 ± 15.9
Mid	217 ± 38.3	204 ± 32.2	171 ± 16.3
Endo	256 ± 57.9	226 ± 43.4	197 ± 27.3
Brain total	51.2 ± 8.55	45.4 ± 8.33	35.4 ± 3.31
Cortex	51.7 ± 2.39	45.5 ± 8.58	34.5 ± 3.29
Cerebellum	51.1 ± 8.48	43.7 ± 7.00	37.7 ± 3.17
Brainstem	49.9 ± 10.3	45.8 ± 8.62	36.8 ± 3.80
Kidneys	251 ± 29.1	262 ± 27.5	233 ± 21.4
Adrenals	670 ± 84.4	606 ± 79.2	458 ± 66.9
Stomach	17.5 ± 1.81	13.8 ± 2.25	21.5 ± 2.90
Small intestine	39.1 ± 5.15	31.3 ± 7.69	55.0 ± 14.0
Liver	62.5 ± 8.50	60.6 ± 10.0	56.1 ± 11.5
Pancreas	51.2 ± 17.4	22.0 ± 3.97	67.1 ± 15.6
Spleen	144 ± 38.2	73.9 ± 17.0	103 ± 16.5
Muscle	3.52 ± 0.81	2.66 ± 0.72	5.40 ± 2.09
Skin	2.32 ± 0.13	1.78 ± 0.35	1.60 ± 0.16
Lungs	109 ± 25.9	91.1 ± 22.3	120 ± 25.6
		Conductance ($ml\ min^{-1}\ mmHg^{-1}\ 100g^{-1}$)	
Organ	*Placebo*	*PY 108–068*	*Verapamil*
Heart total	1.06 ± 0.20	0.99 ± 0.14	0.79 ± 0.07
Epi	1.28 ± 0.22	1.13 ± 0.18	0.89 ± 0.07
Mid	1.38 ± 0.24	1.29 ± 0.16	1.04 ± 0.08
Endo	1.61 ± 0.36	1.42 ± 0.23	1.18 ± 0.14
Brain total	0.33 ± 0.06	0.28 ± 0.04	0.22 ± 0.02
Cortex	0.33 ± 0.06	0.29 ± 0.04	0.21 ± 0.02
Cerebellum	0.33 ± 0.06	0.28 ± 0.04	0.23 ± 0.02
Brainstem	0.31 ± 0.06	0.29 ± 0.05	0.22 ± 0.03
Kidneys	1.60 ± 0.20	1.74 ± 0.18	1.42 ± 0.11
Adrenals	4.18 ± 0.48	3.83 ± 0.36	2.81 ± 0.39
Stomach	0.11 ± 0.01	0.09 ± 0.02	0.13 ± 0.01
Small intestine	0.25 ± 0.04	0.21 ± 0.05	0.33 ± 0.09
Liver	0.39 ± 0.05	0.40 ± 0.06	0.33 ± 0.07
Pancreas	0.31 ± 0.11	0.15 ± 0.04	0.39 ± 0.08
Spleen	0.93 ± 0.29	0.50 ± 0.13	0.64 ± 0.11
Muscle	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01
Skin	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Lungs	0.71 ± 0.18	0.61 ± 0.15	0.74 ± 0.15

The changes from these values are shown in Figures 4, 5, 6 and 7. Abbreviations are as for Table 1. The results are expressed as mean ± s.e. mean and $n = 9$ for each group of measurements.

spheres represents nutritional flow but not arteriovenous shunt flow and this flow was also markedly decreased. Not only was the conductance of, but also the blood flow to, these organs decreased. A modest degree of vasoconstriction was seen in the stomach, the spleen and in the subepicardial and middle layer of the left ventricular free wall, where conductance decreased and flow remained unchanged. No change in conductance was seen in the inner layer of the left ventricular free wall, in the brain or in the skeletal muscle, and hence flow tended to increase. Angiotensin II thus redistributed a slightly reduced cardiac output in favour of the brain, the adrenals, skeletal muscle, the subendocardial layer of the

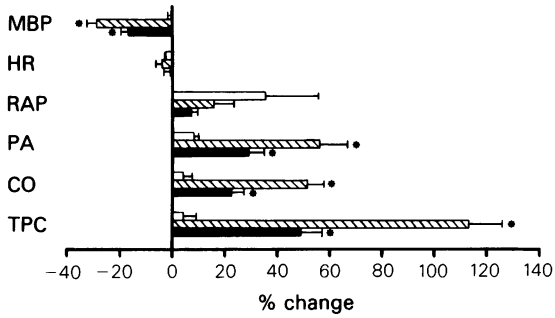

Figure 4 Effects of PY 108–068 (hatched columns, verapamil (solid columns) or placebo (open columns) on systemic haemodynamic variables in animals continuously infused with angiotensin II (A II), 0.15 μg kg^{-1} min^{-1}. Pretreatment values in Table 2, abbreviations as in Table 1. $^*P < 0.05$, significantly different from placebo-treated animals, $n = 9$ in both groups and for all measurements. The vertical bars show s.e. mean.

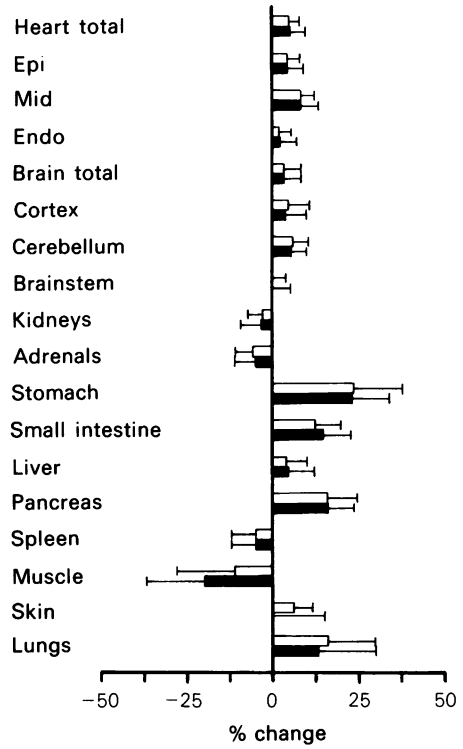

Figure 5 Changes in the peripheral circulation (blood flow (open columns) and peripheral conductance (solid columns) during continuing infusion of angiotension II (A II) and after the administration of vehicle (placebo). Note that the scale is larger than in figures 6 and 7 where the effects of PY 108–068 and verapamil are shown. Abbreviations as in Table 1. The vertical bars show s.e. mean, $n = 9$.

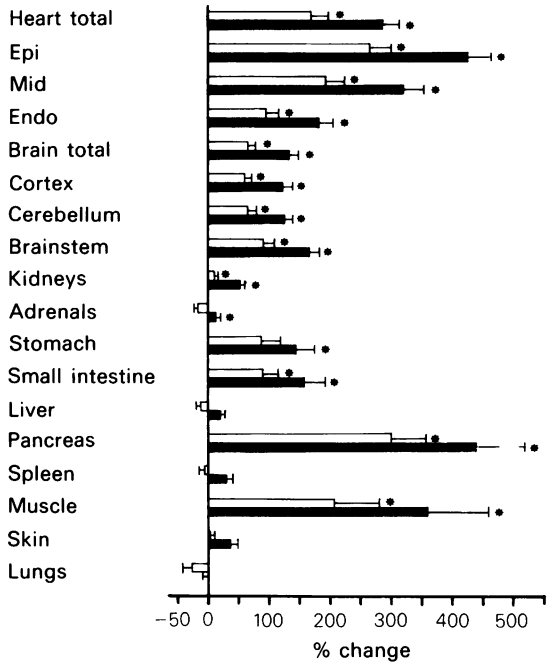

Figure 6 Effects of PY 108–068, 30 μg kg^{-1} i.v. on the peripheral circulation (blood flow (open columns) and peripherals conductance (solid columns)) of animals continuously infused with angiotensin II (A II), 0.15 μg kg^{-1} min^{-1}. $^*P < 0.05$ significantly different from the placebo treated group thus showing the effects of PY 108–068. The vertical bars show s.e. mean, $n = 9$.

heart, and, perhaps (effect not significant) the pancreas.

In Table 2 the systemic haemodynamic, regional flow and conductance values before the administration of the placebo or active substances are shown.

The changes of the systemic haemodynamic variables induced by the infusion of placebo PY or V are shown in Figure 4. Both PY and V decreased mean blood pressure significantly whereas it remained elevated in the control animals under the influence of the A II infusion which was continuous in all the groups. Heart rate remained unchanged in all groups. Right atrial pressure continued to increase in the control groups (as in the previous 10 min period, see Figure 2). This increase tended to be attenuated in treated groups but none of the effects was significant. Peak acceleration, cardiac output and total peripheral conductance increased significantly in the treated cats only.

As shown in Figure 5, there were no significant changes in regional blood flow or conductance in the group infused with the placebo solution. However, the small (but insignificant) changes may have had some influence on the results of the Kruskal Wallis

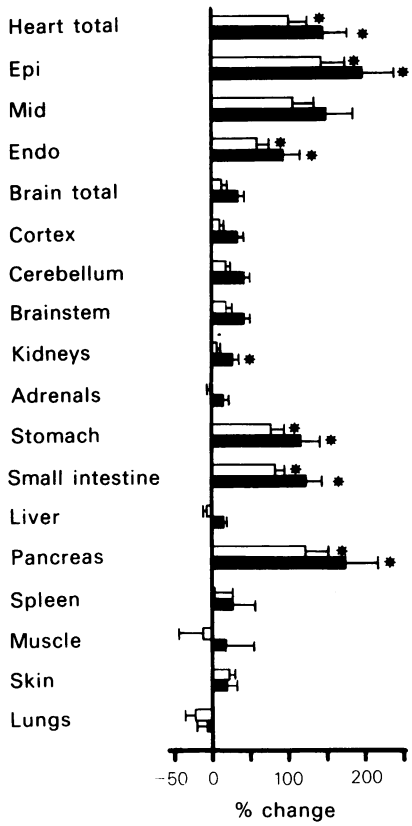

Figure 7 Effects of verapamil, 300 $\mu g\,kg^{-1}$ i.v. on the peripheral circulation (blood flow (open columns) and conductance (solid columns)) continuously infused with angiotensin II (A II), 0.15 $\mu g\,kg^{-1}\,min^{-1}$. $^*P < 0.05$ significantly different from the placebo treated group thus showing the effects of verapamil. The vertical bars show s.e. mean, $n = 9$.

test which was used to compare the changes in blood flow to all parts of the heart and brain occurring during placebo treatment with those induced by PY or V. Blood flow to the kidneys was increased as was blood flow to skeletal muscle, pancreas and small intestine (Figure 6) in the PY-treated group. There was also a tendency for the blood flow to the stomach to increase. This effect did not reach statistical significance, since a tendency towards an increase in blood flow also occurred in the control cats. It is noteworthy, that the vasoconstriction in the liver, the spleen, the skin and the arterio-venous shunts (microspheres in the lungs) was not attenuated by PY. This is shown even more clearly by the effects of PY on organ conductance.

The effects of V (Figure 7) in the third group of cats infused with A II were similar to those of PY on most vascular beds. The magnitude of the changes were smaller because the dose of verapamil was limited by cardiac side effects. In fact in one cat the infusion was stopped at 150 $\mu g\,kg^{-1}$ total dose because of severe bradycardia and cardiac depression. Since the cat recovered rapidly after cessation of infusion and since the direction of the haemodynamic changes in this experiment agreed with the other ones, this cat was included in the data analysis.

In contrast to PY, V did not increase blood flow to skeletal muscle, a vascular bed that was not constricted by A II (Figure 3). No significant effects on cerebral and adrenal flows and conductances were found, but the changes tended to be in the same direction as those observed in the PY-treated cats.

Discussion

Angiotensin receptors occur on cells in many organs and systems (Barer, 1961; Regoli *et al.*, 1974; Devynck & Meyer, 1978; Peach, 1981). Among these are systems involved in the control of blood pressure such as vascular smooth muscle and the adrenal cortex where aldosterone production is stimulated (Deheneffe *et al.*, 1976; Ichikawa *et al.*, 1979; Schiffrin *et al.*, 1981). The mechanism by which these effects are elicited appears to include the induction of action potentials associated with the opening of slow channels (Peach, 1981). The actions of A II on vascular smooth muscle appear to be complex. Most but not all (Weston & Golenhofen, 1976) blood vessels are contracted by A II and this is accompanied by depolarization (Keatinge, 1966; Somlyo & Somlyo, 1968; Healy & Bohr, 1977). Depolarization opens voltage sensitive calcium channels, which are readily blocked by calcium antagonists (Bolton, 1979). Furthermore A II releases calcium from an intracellular store (Deth & Van Breemen, 1974; 1977; Healy & Bohr, 1977) Our own previous work has shown that A II allows calcium to enter the cells of rabbit aorta and elicit a contraction. This effect was only minimally antagonized by PY or verapamil (Hof *et al.*, 1982b). We therefore concluded that the calcium influx observed in the presence of A II could not be explained entirely by depolarization induced activation of potential sensitive channels of the type that are readily blocked by these calcium antagonists in rabbit aorta, and postulated that there were also receptor operated calcium channels that are relatively insensitive to calcium-antagonists.

There is evidence that calcium antagonists may influence the vasoconstrictor effects of A II in man as well as in experimental animals. In volunteers the pressor effects of A II infusion were significantly attenuated by a single dose of the dihydropyridine derivative nifedipine in 2 trials (Miller *et al.*, 1981; Vierhapper & Waldhaeusl, 1982). In another trial, in

which nifedipine was given orally for 2 weeks, such effects could not be shown, but due to the long time interval between the test infusion of A II before and after the nifedipine treatment the variation was large (Beretta-Piccoli *et al.*, 1982).

In animal experiments (pithed rats, dogs) it has been demonstrated that the pressor effect of A II can be diminished by diltiazem and verapamil (Greenberg & Wilson, 1974; Cavero & Lefevre-Borg, 1981). The vasoconstrictor effect of A II on afferent and efferent arterioles of the rat glomerulus was also antagonized by verapamil and the inorganic calcium antagonist manganese (Ichikawa *et al.*, 1979).

The effect of A II on aldosterone secretion by isolated glomerulosa cells is blocked by calcium antagonists (Devynck & Meyer, 1978; Schiffrin *et al.*, 1981) and aldosterone secretion may also be inhibited in man after therapeutic doses of nifedipine (Millar *et al.*, 1981; Vierhapper *et al.*, 1982).

The results of human and whole animal experiments show that calcium antagonists reduce the vasoconstrictor effect of A II on resistance vessels. The object of this study was to see (a) whether this interaction occurred in all vascular beds or showed selectivity and (b) whether the distribution of the vasodilatation produced by the two calcium antagonists acting in the presence of A II differed from that seen in cats not pretreated with the vasoconstrictor agent.

We examined the effects of A II on specific organ vascular beds using the microsphere method. With this method blood flow to an organ is measured and conductance can be calculated. Since large vessels, which are normally used for experiments *in vitro* do not usually limit blood flow, we mostly measured the responses of small resistance vessels to the agents administered.

A II diminished strongly blood flow to the kidneys, the liver and other organs of the intestinal system. The most striking effects occurred in the skin, where both nutritional flow (as expressed by the microspheres trapped in the skin itself) and arterio-venous shunt flow (which also mostly occurs in the skin) were both decreased. No vasoconstriction was found in the brain and in skeletal muscle. A relatively modest decrease in conductance not sufficient to decrease blood flow, occurred in the heart.

Effects of A II similar to those observed in our experiments on cats have been demonstrated in dogs with regard to coronary flow, iliac flow (muscle plus skin), renal flow and blood flow to the brain (Greenberg & Wilson, 1974; Heyndricks *et al.*, 1976; Santamore *et al.*, 1981). The distribution of cardiac output measured using the microsphere technique in rats gave results comparable with ours with respect to the kidneys, but different with respect to most of the other organs measured (Morfaux & Bralet, 1977). This may be due not only to species differences but also to the rather important technical differences between their method and ours.

PY and V reduced the blood pressure elevated by A II back to or, especially in the experiments with PY, slightly below the initial value. The attenuation of the effects of A II on the various regional vascular beds was very selective. The constriction of the arterio-venous shunts was not affected at all and there was only a slight tendency for an attenuation of the strong vasoconstriction occurring in the skin.

Not surprisingly, PY and V caused a marked increase in blood flow and conductance in those vascular beds, where they also caused vasodilatation when administered to the same cat preparation but without any vasoconstrictor pretreatement, namely heart, brain and skeletal muscle (Hof *et al.*, 1982a; Hof, 1983). However, it is most interesting to note that, in the presence of A II, PY and V caused vasodilatation in vascular beds where neither these two nor several other calcium antagonists normally have any effect, namely kidney, liver, and small intestine. The resistance vessels of these organs were strongly constricted by A II and this constriction was reduced significantly in the animals treated with either PY or V.

So far, we have only discussed the mechanism of action of A II on its receptors mediating constriction of smooth muscle cells directly. A II also elicits other effects which might influence the peripheral circulation in acute experiments such as ours. There is evidence that A II may facilitate the liberation of noradrenaline from and inhibit its uptake into nerve endings thus potentiating effects of this neurotransmitter (Malik & Nasjletti, 1976; Zimmermann, 1978; Campbell & Jackson, 1979). We have, however, found in earlier experiments (Hof, 1983) that calcium antagonists had hardly any effect on blood flow to many organs with densely innervated vessels such as the kidneys, the spleen, the skin and the small intestine. In some of these organs, such as the kidneys, but not in others (e.g. the spleen) A II had strong effects. This is an argument against a relevant contribution of A II induced noradrenaline release under our experimental conditions.

A II has also been shown to affect prostaglandin synthesis (Trachte & Lefer, 1980). It is difficult to judge how important this mechanism may be to the effects observed in our experiments. Increased local prostaglandin production induced by A II has been found to enhance its vasoconstrictor action in some vascular beds (Trachte & Lefer, 1980) and to attenuate it in others (Gunther & Cannon, 1980). In dogs A II induced strong coronary vasoconstriction only after pretreatment with indomethacin, which suppressed the A II-induced increase in prostaglandin E_2 synthesis (Gunther & Cannon, 1980). If this also

applies to cats, then it would explain why hardly any vasoconstriction was found in the coronary vascular bed under our experimental conditions.

These considerations, however, do not change the conclusions emerging from our experiments. Two calcium antagonists of completely different chemical structure attenuated selectively the AII induced vasoconstriction in some vascular beds but not in others such as the skin, the arterio-venous shunts and the spleen. The effects were not limited to vascular beds which are dilated under normal circumstances, that is in animals not pretreated with a vasoconstrictor. These vascular beds, the heart, the brain and skeletal muscle are normally under autoregulatory control, a mechanism which appears to be very susceptible to the effects of calcium antagonists. With the mechanism of smooth muscle activation used in the present experiments a different set of vascular beds was sensitive to the effects of calcium antagonists. This implies that the site of action of these two and possibly also of other calcium antagonists might depend on the mechanism of activation of the vessel prevalent at the time of the investigation.

I am grateful to Mr T.D. Lindsay for skilful technical assistance during parts of the experiments.

References

BARER, G.R. (1961). A comparison of the circulatory effects of angiotensin, vasopressin and adrenaline in the anaesthetized cat. *J. Physiol.*, **156**, 49–66.

BERETTA-PICCOLI, C., BIANCHETTI, M.G., WEIDMANN, P., BOEHRINGER, K., LINK, L. & MORTON, J.J. (1982). Effects of acute mild hypercalcemia or of the calcium antagonist nifedipine or cardiovascular responsiveness to norepinephrine or angiotensin II in normal humans. *J. cardiovasc. Pharmac. (Suppl.)*, **4**, 306–312.

BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

CAMPBELL, W.B. & JACKSON, E.K. (1979). Modulation of adrenergic transmission by angiotensins in the perfused rat mesentery. *Am. J. Physiol.*, **236**, 211H–217H.

CAVERO, I. & LEFEVRE-BORG, F. (1981). Effects of diltiazem and verapamil on pressor responses mediated by stimulation of α_1-, α_2- adrenoceptors, angiotensin II and 5-hydroxytryptamine receptors in pithed rats. *Br. J. Pharmac.*, **74**, 839P–840P.

CURTISS, C., COHN, J.N., VROBEL, T. & FRANCIOSA, J.A. (1978). Role of the renin angiotensin system in the systemic vasoconstriction of chronic congestive heart failure. *Circulation*, **58**, 763–770.

DEHENEFFE, J., CUESTA, V., BRIGGS, J.D., BROWN, J.J., FRASER, R., LEVER, A.F., MORTON, J.J., ROBERTSON, J.I.S., & TREE, M. (1976). Response of aldosterone and blood pressure to angiotensin II infusion in anephric man. Effect of sodium deprivation. *Circulation Res.*, **39**, 183–190.

DETH, R. & VAN BREEMEN, C. (1974). Relative contributions of Ca^{2+} influx and cellular Ca^{2+} release during drug induced activation of the rabbit aorta. *Pflüegers Arch.*, **348**, 13–22.

DETH, R. & VAN BEEMEN, C. (1977). Agonist induced release of intracellular Ca^{2+} in the rabbit aorta. *J. membrane Biol.*, **30**, 363–380.

DEVYNCK, M.A. & MEYER, P. (1978). Angiotensin receptors. *Biochem. Pharmac.*, **27**, 1–5.

FREER, R.J. (1975). Calcium and angiotensin tachyphylaxis in rat uterine smooth muscle. *Am. J. Physiol.*, **228**, 1423–1430.

GLOSSMANN, H. & HORNUNG, R. (1980). Calcium- and potassium-channel blockers interact with alpha-adrenoceptors. *Mol. Cell. Endocrinol.*, **19**, 243–251.

GREENBERG, S. & WILSON, W.R. (1974) Iproveratril: A nonspecific antagonist of peripheral vascular reactivity. *Can. J. Physiol. Pharmac.*, **52**, 266–271.

GUNTHER, S., & CANNON, P.J. (1980). Modulation of angiotensin II coronary vasoconstriction by cardiac prostaglandin synthesis. *Am. J. Physiol.*, **238**, 895H–901H.

HEALY, J.K. & BOHR, D.F. (1977). Angiotensin II responses of rabbit aortic strips compared with fast and slow epinephrine responses. *Proc. Soc. exp. Biol. Med.*, **155**, 495–500.

HEYNDRICKS, G.R. BOETTCHER, D.H. & VATNER, S.F. (1976). Effects of angiotensin, vasopressin and methoxamine on cardiac function and blood flow distribution in conscious dogs. *Am. J. Physiol.*, **231**, 1579–1587.

HOF, R.P. (1983). Calcium antagonists and the peripheral circulation: differences and similarities between PY 108–068, nicardipine, verapamil and diltiazem. *Br. J. Pharmac.*, **78**, 375–394.

HOF, R.P. & HOF, A. (1981). Acceleration of blood in the aorta: a parameter useful for evaluating cardiotonic and afterload reducing substances. *J. Pharmac. Methods*, **6**, 87–95.

HOF, R.P. & HOF, A. (1982). A simple cutter for fresh tissue to facilitate the investigation of intramyocardial blood flow with tracer microspheres in cats and rabbits. *J. Pharmac. Methods*, **7**, 197–205.

HOF, R.P., HOF, A. & NEUMANN, P. (1982a). Effects of PY 108–068, a new calcium antagonist, on general hemodynamics and regional blood flow in anesthetized cats: a comparison with nifedipine. *J. cardiovasc. Pharmac.*, **4**, 352–362.

HOF, R.P., HOF, A., SALZMANN, R. & WYLER, F. (1981). Trapping and intramyocardial distribution of microspheres with different diameters in cat and rabbit hearts in vitro. *Basic Res. Cardiol.*, **76**, 630–638.

HOF, R.P., VUORELA, H.J. & NEUMANN, P. (1982b). PY 108–068, a new, potent, and selective inhibitor of calcium-induced contraction of rabbit aortic rings. *J. cardiovasc. Pharmac.*, **4**, 344–351.

HOF, R.P., WYLER, F. & STALDER, G. (1980). Validation studies for the use of the microsphere method in cats and young minipigs. *Basic Res. Cardiol.*, **75**, 747–756.

ICHIKAWA, I., MIELE, J.F. & BRENNER, B.M. (1979). Reversal of renal cortical actions of angiotensin II by verapamil and manganese. *Kidney Int.*, **16**, 137–147.

KARLBERG, B.E., ASPLUND, J., WETTRE, S., OEHMAN, K.P. & NILSSON, O.R. (1982). Long-term experience of captopril in the treatment of primary (essential) hypertension. *Br. J. clin. Pharmac.*, **14,** 133S–137S.

KARLINER, J.S., MOTULSKI, DUNLAP, J., HELLER-BROWN, J. & INSEL, P.A. (1982). Verapamil competitively inhibits alpha$_1$-adrenergic and muscarinic but not beta-adrenergic receptors in rat myocardium. *J. cardiovasc. Pharmac.*, **4,** 515–520.

KEATINGE, W.R. (1966). Electrical and mechanical responses of vascular smooth muscle to vasodilator agents and vasoactive polypeptides. *Circulation Res.*, **18,** 641–649.

LEVINE, T.B. & COHN, J.N. (1982). Factors predicting acute and long-term response to captopril therapy in congestive heart failure. *Br. J. Clin. Pharmac.*, **14,** 169S–174S.

MALIC, K.U. & NASJLETTI, A. (1976). Facilitation of adrenergic transmission by locally generated angiotensin II in rat mesenteric arteries. *Circulation Res.*, **38,** 26–30.

MILLAR, J.A., McLEAN, K. & REID, J.L. (1981). Calcium antagonists decrease adrenal and vascular responsiveness to angiotensin II in normal man. *Clin. Sci.*, **61,** 65s–68s.

MORFAUX, C. & BRALET, J. (1977). Etude des circulations regionales chez le rat par utilisation de microspheres. Influence de la noradrenaline, de l'angiotensine et de la vasopressine. *J. Pharmac.*, **8,** 261–268.

MORRIS, B.J., DAVIS, J.O., ZATZMAN, M.L. & WILLIAMS, G.M. (1977). The renin-angiotensin-aldosterone system in rabbits with congestive heart failure produced by aortic constriction. *Circulation Res.*, **40,** 275–282.

PEACH, M.J. (1981). Molecular actions of angiotensin. *Biochem. Pharmac.*, **30,** 2745–2751.

REGOLI, D., PARK, W.K. & RIOUX, F. (1974). Pharmacology of angiotensin *Paediat. Res.*, **26,** 69–123.

RUDOLPH, A.M. & HEYMANN, M.A. (1967). The circulation of the fetus in utero: Methods for studying distribution of blood flow, cardiac output and organ blood flow. *Circulation Res.*, **21,** 163–184.

SANTAMORE, W.P. BOVE, A.A., CAREY, R., WALINSKY, P. & SPANN, J.F. (1981). Synergistic relation between vasoconstriction and fixed epicardial vessel stenosis in coronary artery disease. *Am. Heart J.*, **101,** 428–434.

SCHAPER, W., LEWI, P., FLAMENG, W. & GIJPEN, L. (1973). Myocardial steal produced by coronary vasodilation in chronic coronary artery occlusion. *Basic Res. Cardiol.*, **68,** 3–20.

SCHIFFRIN, E.L., LIS, M., GUTKOWSKA, J. &. GENEST, J. (1981). Role of Ca^{2+} in response of adrenal glomerulosa cells to angiotensin II, ACTH, K^+, and ouabain. *Am. J. Physiol.*, **241,** 42E–46E.

SCHOLTYSIK, G. & SCHAAD, A. (1983). Cardiac cellular electrophysiology as a tool to prove Ca^{++} slow channel inhibition by PY 108–068. *Triangle*, **22,** 49–55.

SOMLYO, A.V. & SOMLYO, A.P. (1968). Electromechanical and pharmaco-mechanical coupling in vascular smooth muscle. J. Pharmac. exp. Ther., **159,** 129–145.

STUMPE, K.O., OVERLACK, A., KOLLOCH, R. & SCHREYER, S. (1982). Long-term efficacy of angiotensin-converting-enzyme inhibition with captopril in mild-to-moderate essential hypertension. *Br. J. clin. Pharmac.*, **14,** 121S–126S.

TRACHTE, G.J. & LEFER, A.M. (1980). Prostaglandin-mediated angiotensin-induced vasoconstriction in isolated cat arteries. *Blood Vessels*, **17,** 196–201.

VIERHAPPER, H. & WALDHAEUSL, W. (1982). Reduced pressor effect of angiotensin II and of noradrenaline in normal man following the oral administration of the calcium-antagonist nifedipine. *Eur. J. clin. Med.*, **4,** 263S–267S.

WESTON, A.H. & GOLENHOFEN, K. (1976). Comparison of the excitatory and inhibitory effects of angiotensin and vasopressin on mammalian portal vein. *Blood Vessels*, **13,** 350–360.

ZIMMERMANN, B.G. (1978). Actions of angiotensin on adrenergic nerve endings. *Fedn. Proc.*, **37,** 199–202.

(*Received June 3, 1983.*)