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Modification of vasopressin- and angiotensin II-induced changes by calcium antagonists in the peripheral circulation of anaesthetized rabbits

Robert P. Hof

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

1 Investigations into the site of vasodilator and antivasoconstrictor activity of calcium antagonists previously performed in cats were extended to a second species, barbiturate-anaesthetized rabbits, and a second vasoconstrictor agent, vasopressin.

2 The dihydropyridine derivative darodipine (code name PY 108–068; 10, 30 and 100 $\mu g\,kg^{-1}$ i.v.) showed systemic haemodynamic effects comparable to those seen in cats at half these doses. Darodipine effected regional vasodilatation (measured with tracer microspheres) in the heart, brain and skeletal muscles as in cats. Only the vessels of the adrenals (dilated in rabbits but not in cats), and the kidneys and skin (constricted in rabbits but not in cats) responded differently to darodipine.

3 Angiotensin II (A II; 0.15 and 1.5 $\mu g\,kg^{-1}\,min^{-1}$) constricted the same vascular beds in rabbits as in cats, namely the heart, kidneys, small intestine, pancreas, spleen, skin and arterio-venous shunts (inferred from microspheres reaching the lungs), the only exceptions being the vessels of the stomach and liver (constriction only in cats) and the adrenals (constriction only in rabbits).

4 Darodipine (30 and 100 $\mu g\,kg^{-1}$) attenuated the A II-induced vasoconstriction in the same vascular beds in rabbits as in cats including the kidneys, which were constricted after administration of the antagonist alone.

5 These results indicate surprisingly small species differences for the vasodilator effects of darodipine as well as the attenuation of the vasoconstrictor effects of A II.

6 Lysine-vasopressin (2 and 50 mu $kg^{-1}\,min^{-1}$) did not increase blood pressure in anaesthetized rabbits but dose-dependently lowered heart rate, cardiac output, total peripheral conductance and myocardial contractile force (measured with a strain gauge). Vasopressin constricted all peripheral vascular beds dose-dependently, except for those of the kidney and liver.

7 The effects of vasopressin persisted in the animals infused with placebo solution. Darodipine (30 and 100 $\mu g\,kg^{-1}$), but not verapamil (300 and 1000 $\mu g\,kg^{-1}$) reversed the vasopressin-induced cardiac depression and decrease in cardiac output. This probably also explains most of the apparent differences between the effects of the two calcium antagonists on the peripheral circulation.

8 Both calcium antagonists diminished the vasopressin constriction in most vascular beds except those of the spleen, skin and arterio-venous shunts. Most of the effects were dose-related but not strictly competitive, as far as this can be judged based on two doses of agonist and antagonist.

9 As with A II the effects of vasopressin were diminished in vascular beds not normally dilated by calcium antagonists.

10 Calcium antagonists display two typical patterns of activity. The vasodilator pattern consists of dilatation of the vesels of the heart, brain and, to a degree varying with the agents, skeletal muscle. The antivasoconstrictor effects occur in some but not all of the vessels constricted by the constrictor agent, vasoconstriction of the spleen, skin and arterio-venous shunts being resistant to the action of calcium antagonists. The pattern of antivasoconstrictor activity appears to depend on the constrictor compound used, inasmuch as such agents constrict different vascular beds.

Introduction

Activation of various receptors on blood vessels may open receptor operated channels admitting calcium into smooth muscle cells thus eliciting contraction or contributing to it (Bolton, 1979). The interaction of calcium antagonists with effects mediated by receptor stimulation varies considerably depending on the

vessel and on the species studied (Cauvin *et al.*, 1983). Most of these experiments were carried out on isolated vessels *in vitro*. In earlier experiments in whole animals we have searched for vascular beds where calcium antagonists could abolish or diminish angiotensin II (A II)-induced vasoconstriction. We found that the antivasoconstrictor effects of the calcium antagonists were seen even in some vascular beds which were not dilated when calcium antagonists were administered to cats not pretreated with A II (Hof, 1983; 1984). We have speculated that the anatomical site of action of calcium antagonists might change depending on the mechanism contracting the vessel at the time of observation.

The main aim of the present experiments was to extend our investigations to a second species, the rabbit, and to a second vasoconstrictor agent, vasopressin, in order to strengthen or to reject the hypothesis mentioned above. These experiments should also provide a better basis for eventual extrapolations to further species, especially man.

As in the previous series of experiments we investigated whether the vasoconstrictor effects of A II were antagonized specifically in the vascular beds where vasodilatation was observed upon administration of a calcium antagonist or whether the attenuation occurred in different vascular beds. The same experiments were then carried out using vasopressin as a constrictor agent. This endogenous vasoconstrictor exerts an action on blood vessels which depends on extracellular calcium (Altura & Altura, 1977). Therefore, an interaction with calcium antagonists similar in mechanism to that of A II, yet occurring in different parts of the vascular system would be expected if the hypothesis, that the anatomical site of action of calcium antagonists depends on the mechanism of action, is correct.

Methods

Experimental animals

Large mongrel rabbits (body weight 2.8–4.5 kg) were anaesthetized by injection into an ear vein of 25 mg kg^{-1} pentobarbitone followed by 50 mg kg^{-1} phenobarbitone a few minutes later. The animals were tracheotomized and ventilated with a Loosco (Amsterdam) MK2 infant ventilator. Room air was used and a positive end-expiratory pressure of 2 mmHg was applied as soon as the thorax was opened. The ventilation was started with a rate of 45, a volume of 250 ml kg^{-1} min^{-1} and an inspiratory time of 40% of the respiratory cycle; however, the rate was immediately adjusted to the animals own rate and the volume set to keep the end-expiratory CO_2 between 4.0 and 4.5 volume % (measured continuously with a Gould-Godart capnograph). In addition the arterial blood gases were checked regularly. The anaesthesia was then deepened by a further 50 mg kg^{-1} phenobarbitone. Catheters were placed in the lower abdominal aorta, the inferior vena cava and the right atrium. The left atrium was cannulated by means of a thoracotomy in the left 3rd intercostal space. The aortic root was cleaned of connective tissue and a flowprobe (Narco, 4.0–5.5 mm) fitted onto it. The probe was calibrated *in situ* by the reference flow method at the time of the last microsphere injection. 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 use as an ejection phase parameter of myocardial function (Hof & Hof, 1981b). A thoracotomy in the fifth right intercostal space was used to sew a Walton-Brodie strain-gauge onto the right ventricle parallel to the superficial muscle fibres. The output of all devices was amplified and recorded on a Beckman R612 8-channel recorder.

Microspheres

The use of the microsphere method in our laboratory has been reported in detail previously (Hof *et al.*, 1980; Hof & Hof, 1981a; 1982; Hof, 1983).

In brief: for each determination of regional blood flow we injected about 1.5×10^5 microspheres labelled with one of the following isotopes: ^{125}I, ^{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. The reference sample was withdrawn through the catheter in the aorta at a rate of 6 ml min^{-1} at least 30 s after finishing the injection and flushing of the left atrial catheter. The theoretical accuracy of the determination of cardiac output (CO) approximates ± 2% with this protocol (Hof & Hof. 1981a).

At the end of the experiment the animals were killed with an overdose of pentobarbitone and the organs to be counted were dissected and weighed. Samples of skeletal muscle were obtained from the hindlegs. All other organs mentioned were 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 described in detail elsewhere (Hof & Hof, 1982). The papillary muscles were weighed and counted together with the subendocardial layer. The radioactivity of the samples was counted in a Packard gamma counter (Mod 5921) and the spectra processed on an OKI if-800 Mod. 30 microcomputer according to the method of Rudolph & Heymann (1967) with modifications of the calculations described by Schosser *et al.* (1979).

Experimental protocols

After the preparative procedures were complete the rabbits were allowed to stabilize for approximately 60 min while fresh drug solutions were prepared. Synthetic vasopressin (Lysine-vasopressin, Sandoz) was diluted in 5% glucose to give a dose of 100 mu kg^{-1} ml^{-1} solution. Darodipine was dissolved in a mixture of ethanol and polyethyleneglycol 400, 1 ml each per mg of darodipine. This solution was diluted with 5% glucose to give a dose of 30 $\mu g\,kg^{-1}$ ml^{-1} solution. The same amounts of ethanol and polyethyleneglycol were added to the verapamil solution (300 $\mu g\,kg^{-1}\,ml^{-1}$) so that only one control group was needed. The vasodilator effects of darodipine were investigated according to a protocol used previously (Hof, 1983). Darodipine was infused at 3 different rates during 10 min periods. The doses were 10 + 20 + 70 $\mu g\,kg^{-1}$, so that total cumulative doses of 10, 30 and 100 $\mu g\,kg^{-1}$ were reached at the end of each 10 min infusion period, when measurements were obtained.

In a second series of experiments the interaction between darodipine and A II was investigated. A II was infused continuously at a rate of 0.15 $\mu g\,kg^{-1}$ min^{-1}. Haemodynamic measurements were obtained and microspheres were injected just before and 10 min after the start of the infusion. Then a first dose of darodipine (30 $\mu g\,kg^{-1}$) was infused over 15 min and a third set of measurements was obtained. Up to this point the protocol was comparable to that used previously in cats (Hof, 1984). However, the new experiment was carried further by the infusion of a second dose of darodipine (plus 70 $\mu g\,kg^{-1}$ given over 15 min, to bring the total cumulative dose to 100 $\mu g\,kg^{-1}$ 30 min after starting the administration of darodipine). The A II infusion rate was then increased to 1.5 $\mu g\,kg^{-1}\,min^{-1}$ in order to assess whether the effects of the calcium antagonist could be reversed by this high dose of A II. The final measurements were obtained after 2 min of infusion at this rate.

A preliminary series of experiments indicated that the protocol had to be modified slightly for the vasopressin experiments. Because of the relatively long half-life of vasopressin, a loading dose of 10 mu kg^{-1} was infused during the first 2 min followed by a maintenance infusion of 2 mu $kg^{-1}\,min^{-1}$; 18 rabbits were infused according to this protocol. Haemodynamic measurements were obtained and microspheres were injected just before and 10 min after the start of the infusion. The first dose of darodipine (30 $\mu g\,kg^{-1}$) or verapamil (300 $\mu g\,kg^{-1}$) was then infused for a further 15 min into 6 rabbits each and 6 further animals were infused with the vehicle of the active drugs (placebo) and thus served as the control group. After obtaining measurements at the end of this 15 min period a second dose of the calcium antagonists was infused: plus 70 $\mu g\,kg^{-1}$ darodipine (total cumulative dose 100 $\mu g\,kg^{-1}$) and plus 700 $\mu g\,kg^{-1}$ verapamil (total cumulative dose 1000 $\mu g\,kg^{-1}$). Just before terminating the experiments the infusion of vasopressin was accelerated to 50 mu $kg^{-1}\,min^{-1}$ in order to assess whether or not the effects of the calcium antagonist could be reversed by a higher dose of vasopressin and the last set of measurements was obtained after 2 min of infusion at this rate. Except for the initial loading dose the protocol was thus identical to that of the second series.

Calculations and statistical evaluation

Total peripheral conductance (TPC) was calculated by dividing cardiac output by mean arterial pressure, neglecting the small right atrial pressure. Similarly, the conductance of the peripheral vascular beds was calculated from the regional blood flow and mean blood pressure. The microspheres trapped in the lungs were considered to represent arterio-venous (A-V) shunt flow and no allowance was made for the small contribution of bronchial flow (Warren & Ledingham, 1974). The U-test of Wilcoxon for paired samples was used to assess the effects of darodipine in the first series, the effects of A II and their modification by

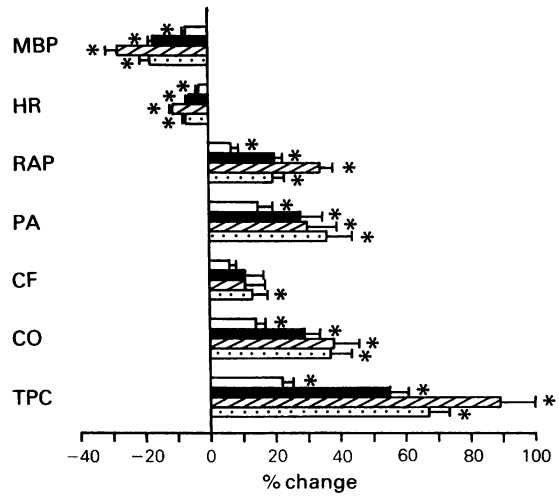

Figure 1 Dose-response relationship for the systemic haemodynamic effects of infusions of darodipine at 3 different rates for 10 min intervals resulting in the following cumulative doses: 10 (open columns), 30 (solid columns) and 100 (hatched columns) $\mu g\,kg^{-1}$. A final measurement was obtained 60 min after the start of the experiment, i.e. 30 min after the end of the last infusion, in order to assess duration of action (stippled columns). Abbreviations as in Table 1. * $P < 0.05$ (Wilcoxon's test), significantly different from base line values (see Table 1). The vertical bars show s.e.mean, $n = 5$.

Table 1 Baseline values for the systemic haemodynamic variables and peripheral conductance, before the administration of either (A) darodipine (vasodilator), (B) angiotensin II or (C) vasopressin

	Systemic variables	*A*	*B*	*C*
HR	(beats min^{-1})	272 ± 7.7	258 ± 5.1	276 ± 4.3
MBP	(mmHg)	69 ± 2.3	76 ± 3.5	76 ± 2.1
RAP	(mmHg)	3.4 ± 0.32	3.5 ± 0.3	3.2 ± 0.2
CF	(g)	28.8 ± 2.87	27.0 ± 3.3	34.6 ± 3.3
CO	($ml\,min^{-1}\,kg^{-1}$)	92 ± 2.7	84 ± 4.3	85 ± 3.4
TPC	($ml\,min^{-1}\,mmHg^{-1}\,kg^{-1}$)	1.34 ± 0.06	1.11 ± 0.06	1.31 ± 0.05
PA	($ml\,s^{-2}$)	444 ± 20.3	460 ± 19.8	430 ± 19.5
	Conductance ($ml\,min^{-1}\,mmHg^{-1}\,100g^{-1}$)			
Organ		*A*	*B*	*C*
Heart total		2.01 ± 0.14	1.51 ± 0.08	1.68 ± 0.10
Epi		2.20 ± 0.19	1.76 ± 0.13	1.86 ± 0.11
Mid		2.59 ± 0.25	2.15 ± 0.16	2.52 ± 0.14
Endo		2.83 ± 0.23	2.37 ± 0.24	2.55 ± 0.16
Brain		0.46 ± 0.03	0.34 ± 0.01	0.42 ± 0.02
Kidneys		3.22 ± 0.41	3.45 ± 0.29	3.83 ± 0.25
Adrenals		2.44 ± 0.39	1.89 ± 0.16	1.83 ± 0.20
Stomach		0.58 ± 0.08	0.42 ± 0.03	0.54 ± 0.05
Small intestine		0.72 ± 0.11	0.71 ± 0.06	0.56 ± 0.05
Liver		0.12 ± 0.02	0.18 ± 0.07	0.27 ± 0.04
Pancreas		0.73 ± 0.11	0.65 ± 0.12	0.80 ± 0.08
Spleen		7.67 ± 0.99	9.07 ± 1.44	7.89 ± 0.98
Muscle		0.06 ± 0.01	0.03 ± 0.004	0.04 ± 0.004
Skin		0.11 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
Lungs		1.02 ± 0.28	0.77 ± 0.13	0.46 ± 0.07

The changes from these values are shown in Figures 1–6. Abbreviations: HR: heart rate; MBP: mean blood pressure; RAP: right atrial pressure; CF: contractile force of the myocardium; TPC: total peripheral conductance; PA: peak acceleration of blood in the aorta; Epi, Mid, Endo: Subepicardial, middle and subendocardial layer of the left ventricular free wall. The results are expressed as mean ± s.e.mean, $n = 5$ (A and B) and $n = 18$ (C).

darodipine in the second series, the vasopressin effects in the preliminary dose-response experiments and the effects of vasopressin in the group of rabbits infused with the vehicle for darodipine and verapamil (placebo).

The effects of the calcium antagonists in the vasopressin-treated animals, as well as the effects of the final administration of vasopressin at the end of the experiment, were evaluated by comparing the changes occurring in the placebo group with the changes induced by darodipine or verapamil using the Kruskal-Wallis and Dunn-Bonferroni test as discussed elsewhere (Hof, 1983); P values <0.05 were considered significant. For the Figures the mean changes were transformed into % changes from base line values.

Results

Vasodilator effects of darodipine

The base line values for all variables and all three series of experiments are shown in Table 1. Darodipine decreased mean blood pressure (MBP) and heart rate (HR) while right atrial pressure (RAP), cardiac output (CO) and total peripheral conductance (TPC) increased and contractile force (CF) tended to increase (Figure 1). All effects were dose-dependent.

As shown in Figure 2, darodipine increased regional vascular conductance dose-dependently in the heart and its regions, brain, skeletal muscle and adrenals. The dose-response relationships indicate different sensitivities to the effects of darodipine for different target tissues. For the coronary arteries and the vessels of skeletal muscle all 3 doses appeared to be on the steep part of the dose-response curves. However, the dilatation of the cerebral vessels reached a plateau with the second dose. It is noteworthy that there was a considerable difference in the response of the subendocardial as compared to the subepicardial vessels, both the magnitude of the changes and the steepness of the dose-response curve being less in the subendocardial layer.

Surprisingly we observed renal vasoconstriction; conductance decreased by 36% at the highest dose. Vasoconstriction also occurred in the spleen and the skin. The 60 min values (stippled columns) indicate

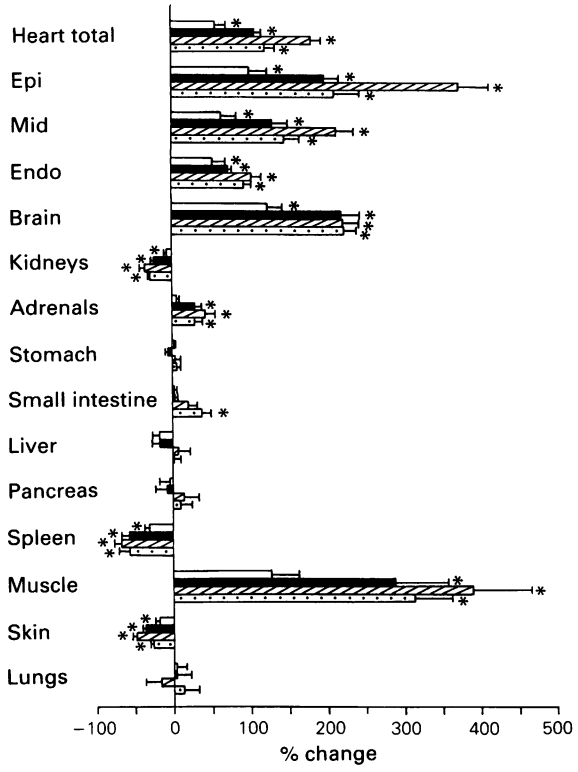

Figure 2 Dose-response relationship for the effects of infusions of darodipine, at 3 different rates (see Figure 1), on regional conductance. The results were obtained from the same experiments as in Figure 1. Abbreviations as in Table 1.

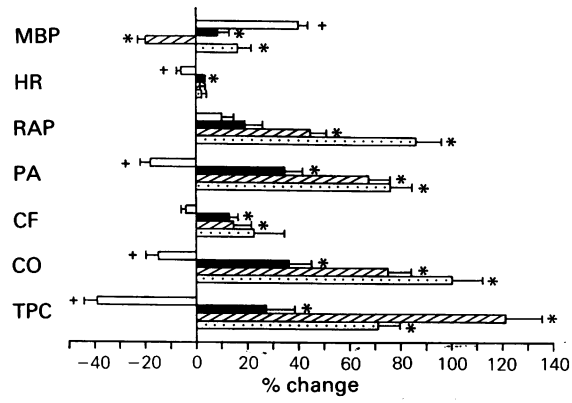

Figure 3 Systemic haemodynamic effects of angiotensin II (AII), infused at a rate of $0.15\,\mu g\,kg^{-1}\,min^{-1}$ (open columns), followed by an additional infusion of darodipine $30\,\mu g\,kg^{-1}$ (solid columns) and $100\,\mu g\,kg^{-1}$ (hatched columns). The stippled columns show the changes induced by accelerating the AII infusion to $1.5\,\mu g\,kg^{-1}\,min^{-1}$. Abbreviations as in Table 1. $^{+}P<0.05$ (Wilcoxon's test), significantly different form base line values (see Table 1). $^{*}P<0.05$, significantly different from values obtained during AII infusion alone (open columns). The vertical bars show s.e.mean, $n = 5$.

that the duration of action of the compound was long enough to justify the use of cumulative doses. Interestingly, the loss of effect with time depended on the target tissue: it was smallest for the vessels of the brain and the subendocardial layer of the heart.

Interaction between angiotensin II *and darodipine*

Table 1 lists the baseline values for this group of 5 rabbits. As shown by the open columns in Figure 3, A II increased MBP strongly and decreased CO causing a correspondingly large decrease in TPC. HR and PA were slightly decreased, CF also tended to decrease. Darodipine dose-dependently reversed all systemic effects of A II (solid and hatched columns), except for RAP which was increased after the high dose of the calcium antagonist. Contrary to the effects of the initial dose of A II, the high dose did not decrease CO and PA (stippled columns). The pattern of effects of A II was thus altered qualitatively by darodipine.

As shown in Figure 4, A II effected sharply differing changes in the various regional vascular beds. Slight vasoconstriction (not accompanied by a decrease in flow) was seen in the heart and small intestine, whereas severe vasoconstriction (decrease in blood flow despite the increased blood pressure) was elicited in the kidneys, adrenals, pancreas, spleen, skin and the lungs (arterio-venous shunt-flow).

The effects of darodipine on the peripheral circulation in these animals continuously infused with A II are also shown in Figure 4 (solid and hatched columns). As expected, strong vasodilatation was observed in the regions of the heart, brain and skeletal muscle. The constrictor effects of A II were fully reversed in the adrenals and the small intestine and attenuated in the kidneys and the pancreas. Darodipine had no effect on the A II-induced constriction in the skin, spleen and of A-V shunts.

Interestingly the high dose of A II (stippled columns) enhanced the effects of darodipine on the vascular beds in the heart, and brain, but reversed them partially or fully in the kidneys, adrenals, pancreas and skeletal muscle. In the skin vasoconstriction was enhanced.

Systemic haemodynamic and regional vasoconstrictor effects of vasopressin

In a preliminary series of experiments, dose-response curves for the systemic haemodynamic vasopressin

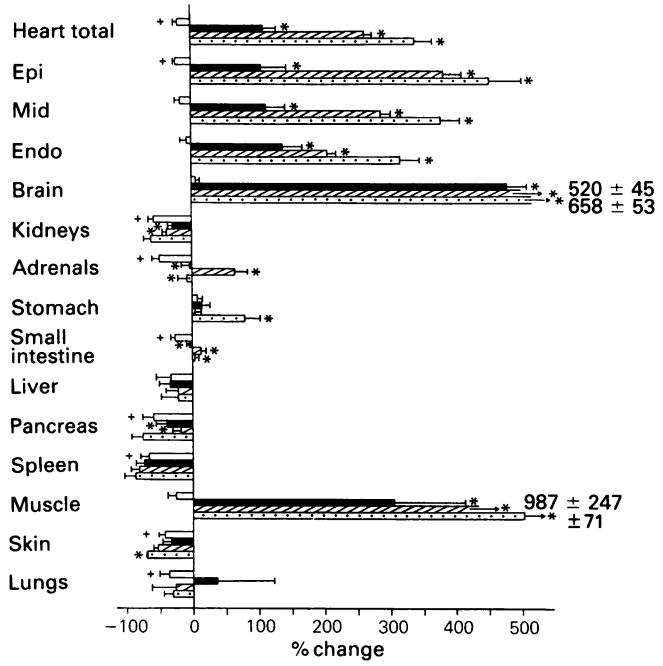

Figure 4 Effects of angiotensin II (AII), 0.15 $\mu g\,kg^{-1}\,min^{-1}$, on regional conductance (open columns) and modification of these effects by darodipine 30 $\mu g\,kg^{-1}$ (solid columns) and 100 $\mu g\,kg^{-1}$ (hatched columns); followed by an accelerated infusion of AII (1.5 $\mu g\,kg^{-1}\,min^{-1}$. The results were obtained from the same experiments as in Figure 3, where details are given in the legend. Abbreviations as in Table 1.

effects were obtained. As shown in Figure 5 all effects were dose-dependent except for the changes in blood pressure and right atrial pressure. For the main series of experiments a dose decreasing TPC by 20–30% (steep part of the dose-response curve) was chosen and because of the relatively long half-life of vasopressin (Montani *et al.*, 1980) we had to use a loading dose followed by a slower maintenance infusion. Our preliminary experiments indicated that a constant effect level could be achieved by a maintenance infusion at 1/5th of the initial rate, once the desired effect had been obtained by the loading dose. Eighteen rabbits were infused according to this protocol and then divided into 3 groups for treatment with placebo

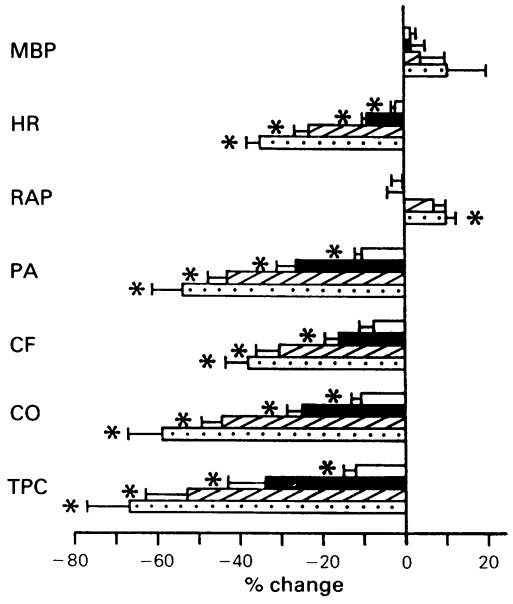

Figure 5 Dose-response relationship for the systemic haemodynamic effects of infusions of vasopressin, 3 (open columns), 10 (solid columns), 30 (hatched columns) and 100 (stippled columns) mu $kg^{-1}\,min^{-1}$, each infusion lasted 2 min and sufficient time was allowed for all variables to return to base line values between infusion periods. Based on this information and the half-life of the effects of vasopressin (estimated from the recovery periods) a protocol, described in Methods, was worked out for the main series of experiments so that effects similar to 10 and 100 mu $kg^{-1}\,min^{-1}$ were obtained. Abbreviations as in Table 1. * $P < 0.05$ (Wilcoxon's test), significantly different from base line values. The vertical bars show s.e.mean, $n = 6$.

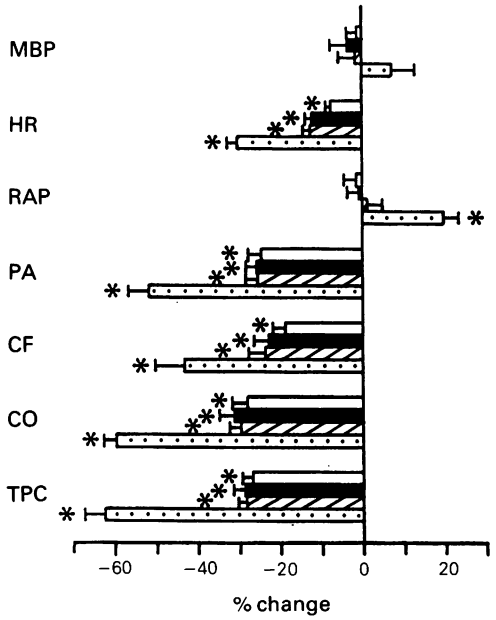

Figure 6 Systemic haemodynamic effects of vasopressin, infused continuously at a rate of 2 mu kg^{-1} min^{-1} (open columns) and 50 mu kg^{-1} min^{-1} (stippled columns), according to a protocol detailed in Methods. The solid and hatched columns represent the changes seen when the infusions of vasopressin (2 mu kg^{-1} min^{-1}) were followed by an infusion of the vehicles (placebo) used for the two infusions of calcium antagonists. Abbreviations as in Table 1. * $P < 0.05$ (Wilcoxon's test), significantly different from base line values. The vertical bars show s.e.mean, $n = 6$.

infusions or calcium antagonists. All effects of the calcium antagonists were determined by comparison with the changes observed in a group infused with the vehicle of the active drugs (placebo group) so that possible alterations in the effects of vasopressin over time were fully respected for statistical evaluation with the Kruskal-Wallis test (Hof, 1983).

There were no differences in the baseline values between the three groups, therefore, pooled values for all 18 rabbits are shown in Table 1. The systemic effects (Figure 6) of vasopressin agree with the corresponding effects in the preliminary experiments (Figure 5). The rate of the constant infusion was obviously well chosen since the effects were indeed constant during the placebo infusion of 1 plus 2.3 ml of the vehicle (solid and hatched columns), except for a slight decrease in HR.

Vasopressin constricted almost all regional vascular beds (Figure 7 and also open columns in Figures 9 and 10) with the exception of the kidneys (significant vasodilatation), liver (no change) and the brain (con-

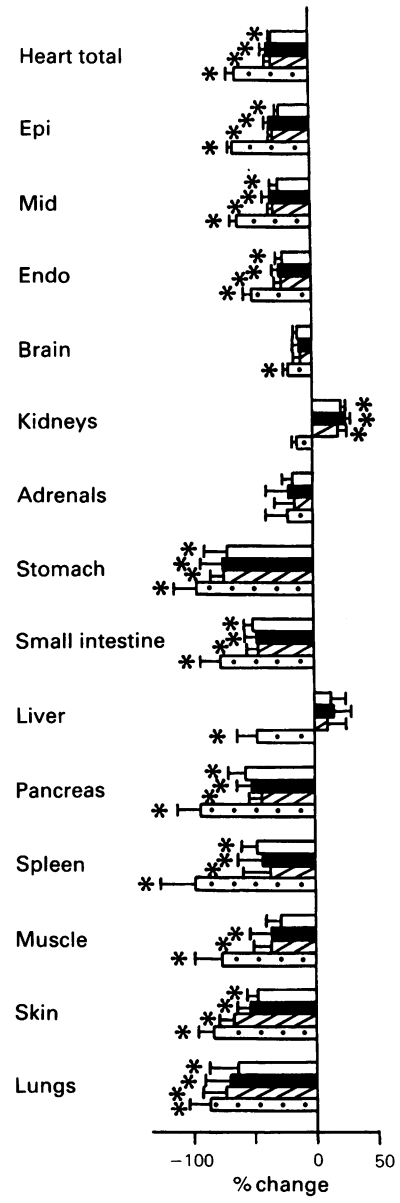

Figure 7 Effects of vasopressin, infused at a constant rate of 2 mu kg^{-1} min^{-1} (open, solid and hatched columns) and a rapid rate of 50 mu kg^{-1} min^{-1} (stippled columns) on regional conductance. The solid and hatched columns represent the effects seen when the vehicles used for the two calcium antagonists were infused (1 plus 2.3 ml) with the vasopressin. The results were obtained from the same experiments as in Figure 6, where details are given in the legend. Abbreviations as in Table 1.

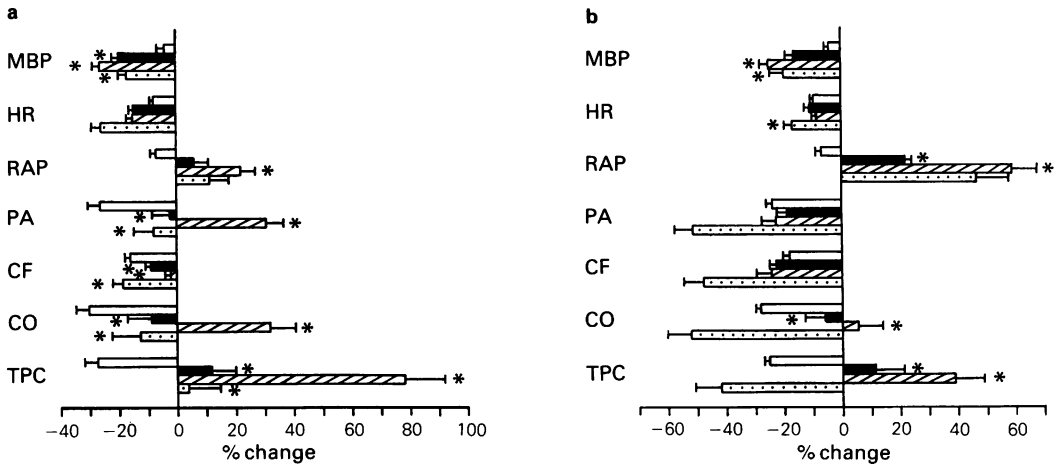

Figure 8 Systemic haemodynamic effects of vasopressin, infused continuously at a rate of 2 mu kg^{-1} min^{-1} (open columns), followed by the additional infusion of either (a) darodipine 30 (solid columns) and 100 (hatched columns) $\mu g\ kg^{-1}$, or (b) verapamil 300 (solid columns) and 1000 (hatched columns) $\mu g\ kg^{-1}$. The stippled columns show how the effects were changed by accelerating the vasopressin infusion to 50 mu kg^{-1} min^{-1}. Abbreviations as in Table 1. * $P < 0.05$ (Kruskal-Wallis and Dunn-Bonferroni test), significantly different from values obtained in placebo-treated animals (see Figure 6). The vertical bars show s.e.mean, $n = 6$.

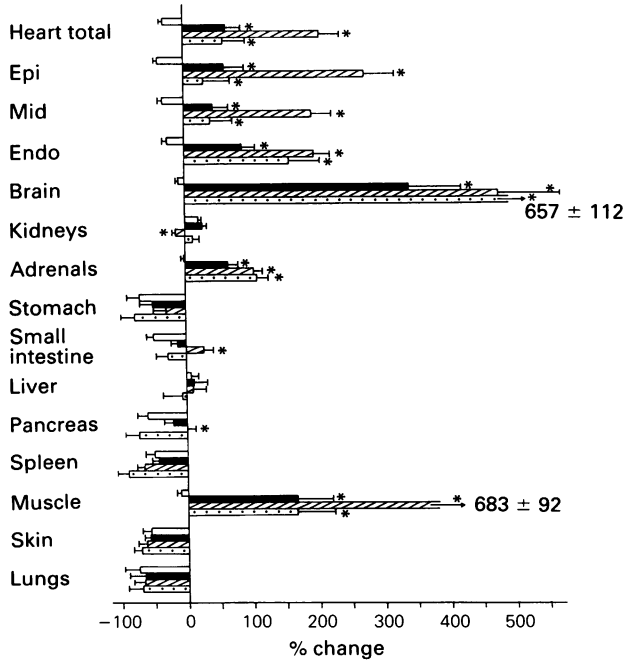

Figure 9 Regional haemodynamic effects of vasopressin 2 mu kg^{-1} min^{-1} (open columns), and their modification by darodipine 30 (solid columns) and 100 (hatched columns) $\mu g\ kg^{-1}$. (stippled columns) Effects seen on accelerating vasopressin infusion to 50 mu kg^{-1} min^{-1}. The results were obtained from the same experiment as in Figure 8a, where details are given in the legend. Abbreviations as in Table 1.

striction only with the high dose). The most prominent effects were elicited in the splanchnic circulation, skin and A–V shunts. The effects were well maintained and the infusion of the placebo solution (the vehicle of the active drugs) obviously had no effect on the various beds as is especially shown by the hatched columns (high rate of infusion of vehicle).

As expected the high rate of vasopressin infusion (stippled columns) caused proportionately more vasoconstriction in the beds constricted by the slow infusion. The high dose also constricted the renal and hepatic arterial beds and had very strong effects on the vasculature in the stomach, small intestine, pancreas and spleen.

In all three groups of animals the initial vasopressin effects (open columns; Figures 8–10) were comparable.

Effects of darodipine and verapamil in the vasopressin-pretreated rabbits

As shown in Figure 8a the systemic haemodynamic effects of darodipine in vasopressin-treated animals were similar to those described in the previous sections. Verapamil (Figure 8b) differed from darodipine mainly with respect to its effects on cardiac function, since the negative inotropic effect of vasopressin was not reversed. The vasopressin-induced decrease in heart rate was not altered by either drug.

The high dose of vasopressin (stippled columns) partly reversed the systemic haemodynamic effects in animals pretreated with darodipine. However, all variables, except HR and RAP, remained significantly different from those of placebo-treated animals. In the verapamil-treated rabbits mean blood pressure remained significantly lower and heart rate significantly higher than in placebo-treated animals. The parameters of myocardial function (PA and CF) as well as CO were depressed to a similar extent as in control animals.

Qualitatively the effects of darodipine (Figure 9) and verapamil (Figure 10) on the regional circulation during vasopressin infusion were similar. Both compounds dilated the vessels of the heart and its regions, the brain and skeletal muscle, in a dose-dependent manner, to values far above the pre-vasopressin base line in values. Comparison of Figure 9 with Figure 2 shows that, firstly, conductance in the above organs reached higher values than in animals not pretreated with vasopressin and, secondly, dilatation was also found in organs not dilated by darodipine alone. This is true for the adrenals, stomach, and small intestine. The constriction of arterio-venous shunt vessels was not diminished by either darodipine or verapamil and the vasconstriction of skin vessels was not attenuated by either calcium antagonist. In the other organs or regions the effects of both calcium antagonists were

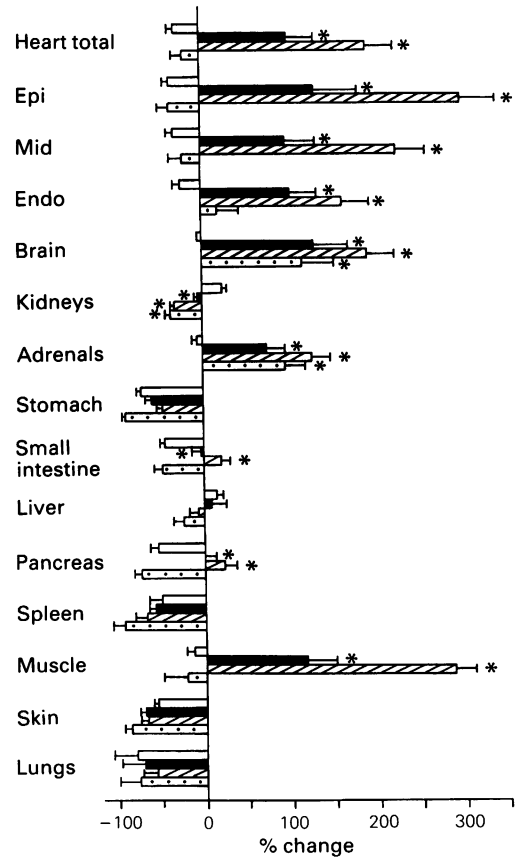

Figure 10 Regional haemodynamic effects of vasopressin, and their modification by verapamil 300 (solid columns) and 1000 (hatched columns) $\mu g\,kg^{-1}$. (stippled columns) Effects seen on accelerating the vasopressin infusion to $50\,mu\,kg^{-1}\,min^{-1}$. The result were obtained from the same experiment as in Figure 8b, where details are given in the legend. Abbreviations as in Table 1.

dose-dependent. The higher dose of darodipine and both doses of verapamil decreased conductance in the kidneys.

The high dose of vasopressin used diminished the effects of darodipine on conductance in the heart and the two outer layers of the left ventricular free wall, the stomach, small intestine, pancreas, spleen, muscle and skin. Vasodilatation was enhanced in the brain. In most regions darodipine induced a significant difference compared to the placebo-treated animals. In contrast, the vasodilator effects of verapamil were diminished or reversed in all regions.

Discussion

Vasodilator effects: species differences between cats and rabbits

Little is known about the effects of calcium antagonists on the systemic and regional circulation in rabbits. Bolt & Saxena (1983) have investigated the effects of the dihydropyridine derivative felodipine in hypertensive rabbits and they observed systemic and regional haemodynamic effects similar to ours with respect to the conductance of heart, brain and skeletal muscle. By contrast they found increases in conductance in the kidneys, skin and stomach. However, they used conscious rabbits with hypertension induced by perinephritis and, moreover, a different calcium antagonist. These factors are probably sufficient to account for the differences.

Since we were interested in species differences, we designed our experiments so that they were as comparable as possible to earlier ones performed on cats (Hof, 1983). The main difference consisted in the type of anaesthesia used (barbiturate instead of chloralose/urethane). Under these conditions most effects of darodipine were comparable in the two species. Differences were observed with regard to the effects of darodipine on the kidneys, the doses required and dose-effect relationships in some vascular beds.

In cats darodipine effected renal vasodilatation just sufficient to compensate for the fall in blood pressure (autoregulation?). In rabbits vasoconstriction occurred. The mechanism for this is unclear though, it may be due to activation of the sympathetic nervous system. A parallel effect was observed in the form of a positive inotropic effect on the heart, as measured by the increase in isometric force development. The functional consequences of this renal vasoconstriction on diuresis have not been investigated yet, but in conscious rats and in man calcium antagonists enhance diuresis (Ene *et al.*, 1984, Narita *et al.*, 1983, Yokoyama & Kaburagi, 1983).

Generally rabbits were less sensitive to the effects of darodipine than cats: about double the dose of darodipine was needed for comparable effects. However, the cerebral circulation was equally sensitive in rabbits and in cats (Hof, 1983).

Vasoconstrictor effects of angiotensin II *and their modification by darodipine in rabbits and cats*

This experiment, like the one discussed above, also served primarily to assess species differences. The protocol was identical in its first part, i.e. the first 3 measuring periods, with the one used in cats (Hof, 1984). Two further measuring periods allowed an assessment as to whether or not the antivasoconstrictor effects of the calcium antagonists were dose-related and whether or not they were reversed by a massively increased dose of the vasoconstrictor. The effects of vasopressin were investigated according to a protocol as similar as possible to that used in a parallel series of experiments so that direct comparisons are possible.

The profile of the A II effects was very similar in rabbits and cats. A II constricted the vessels of the stomach and liver (hepatic artery) only in cats and the vessels of the adrenals only in rabbits.

The effects of darodipine on systemic and regional haemodynamic variables were qualitatively and quantitatively almost indistinguishable from those of darodipine in cats similarly infused with A II. Interestingly darodipine also diminished the A II-induced renal vasoconstriction in rabbits even though, when administered alone, it constricted this vascular bed. This observation gives strong support to the idea that vasodilator and antivasoconstrictor effects appear to rely on different mechanisms. It is tempting to speculate that the vasodilator mechanism is related to an action on potential sensitive channels whereas an effect on receptor operated channels could account for the antivasoconstrictor effects.

The high dose of A II elicited effects which were clearly tissue-dependent. The effects of darodipine were reversed in some vascular beds (kidneys, adrenals, pancreas) but not in others (heart, brain, muscle). Qualitatively similar results were obtained with vasopressin and the conclusions will be discussed below.

There was thus surprisingly extensive agreement (with few exceptions) between the many effects of darodipine, A II and the interaction of the two agents. Extrapolation to other species including man for important target tissues appears to be more justified now since these results confirm a close similarity in two very different species under comparable experimental conditions.

Vasoconstrictor effects of vasopressin in rabbits

The purpose of the present experiments was to study the effects of calcium antagonists on vasopressin-activated vessels. To this end vasopressin (like A II in the corresponding studies) had to dominate over other vasoconstrictor influences possibly active in intact animals. For this reason we used high doses of vasopressin, supposed to cause plasma levels outside the physiological range (Montani *et al.*, 1980).

The effects of vasopressin in our rabbit experiments are in reasonable agreement with those found in other species as far as comparisons are possible between rather different experiments. Barer (1961) obtained similar results in anaesthetized cats and in conscious dogs high doses of vasopressin increased resistance in all vascular beds examined (Heyndrickx *et al.*, 1976); renal vasoconstriction was modest. It should be noted, that in these experiments blood pressure increased by

up to 55%. The infusion of a very small dose of vasopressin into dogs also effected small but widespread decreases of flow, reaching statistical significance for skeletal muscle, heart, brain and skin (Liard *et al.*, 1982). In rats vasopressin again appears to cause very widespread vasoconstriction including the kidneys (Hoffman, 1980). Many older studies in animals and man, reviewed by Saameli (1968), also indicate widespread vasoconstriction which more or less spares the kidneys.

The differences between A II- and vasopressin-induced vasoconstriction can be summarized as follows: (1) vasopressin elicits a much more general vasoconstriction than A II, even though blood pressure effects are trivial or lacking. Only the renal and the hepatic arterial vessels were not constricted, whereas the more selective A II spared the heart, brain, stomach and skeletal muscle. Interestingly, vasopressin favoured exactly two organs strongly affected by A II, the kidneys and liver. (2) The blood pressure response can be used as an indicator of the vasoconstrictor effects of A II but bears no relationship to the vasoconstrictor effects of vasopressin. The interaction of vasopressin with the baroreceptor reflex probably explains this fact (Cowley *et al.*, 1974; Heyndrickx *et al.*, 1976; Liard *et al.*, 1981; Rascher *et al.*, 1981; 1983). (3) Myocardial function is depressed by vasopressin but not by A II. In our experiments cardiac depression contributed to the decrease in cardiac output. The consequences for blood flow to the vascular periphery have not been considered adequately in the past.

Modification of the vasopressin-induced vasoconstriction by darodipine and verapamil

Darodipine and verapamil, when administered to rabbits receiving a continuous infusion of vasopressin, elicited systemic effects similar to those found without vasoconstrictor pretreatment. With darodipine a detailed comparison (Figures 1 and 2) is possible and shows some differences in the dose-response relationship: in Figure 2 the step from 30 to 100 $\mu g\,kg^{-1}$ induced only small changes, showing that for most variables the top of the dose-response curve had been reached. This was not so during the vasopressin infusion when the higher dose induced massive increases especially of CO and TPC.

Verapamil has relatively prominent cardiodepressant effects compared to darodipine (Hof & Scholtysik, 1983). This may be the reason why only darodipine reversed the vasopressin-induced cardiac depression. The difference between the CO in the darodipine- and verapamil-treated animals probably explains most of the differences observed in the peripheral circulation especially after the high dose of vasopressin when CO was very low in verapamil-treated animals.

The two doses of constrictor agent and of calcium antagonist used allow a guess as to the nature of the interaction between the two agents. That the interactions were neither simply additive nor simply competitive nor uniform for all vascular regions in the sense of agonism and antagonism can be seen with both calcium antagonists, especially clearly with darodipine. The effects of the vasoconstrictors and the calcium antagonists were not simply additive: the solid or hatched columns of Figure 2 plus the corresponding columns of Figure 7 (effect of darodipine alone plus effect of vasopressin alone) do not add up to the effects seen in Figure 9. This is especially visible for the vessels of the stomach, small intestine and pancreas where darodipine alone had no effect.

That the interaction was not of a simple competitive type, at least not in all vascular beds, is best appreciated from the effects on the brain vessels. The slow infusion of vasopressin constricted these vessels; however, the high dose increased conductance beyond the increase elicited by the high dose of darodipine. A II also enhanced the increase in conductance in the brain (and some regions of the heart) after darodipine treatment. A non-competitive interaction should be expected, since clearly calcium antagonists do not compete with the vasoconstrictor agents for the same receptor (Bolton 1979; Cauvin *et al.*, 1983).

As with A II, calcium antagonists attenuated the constrictor effects of vasopressin only in some and not in other vascular beds such as the spleen and skin. It thus appears, that in these organs the vessels that control conductance, i.e. the arterioles, are not sensitive to calcium antagonists, whether they are constricted by A II or by vasopressin. This is also true for the A-V shunt vessels. In a different series of experiments where the interaction between ouabain and calcium antagonists was investigated (Hof & Hof, 1985), we have observed that the constrictor effects of this agent on the spleen, skin and arterio-venous shunts were also resistant to darodipine and verapamil.

This brings to mind experiments *in vitro* on rabbit facial veins (Winquist & Baskin, 1983) or aorta (Hof *et al.*, 1982b), where different calcium channels are found, some of which are, and others which are not, blocked by organic calcium antagonists.

We can thus distinguish between three types of interactions between darodipine (and presumably other calcium antagonists) and both A II and vasopressin mediated vasoconstriction: (1) no modification (e.g. spleen, skin); (2) an apparently competitive interaction as far as our experiments allow such a statement (e.g. pancreas, kidneys) and (3) a clearly non-competitive behaviour (e.g. vasculature of the brain). This indicates that receptor activation can mobilize calcium in several very different ways depending on the blood vessels considered. It is therefore

likely that extrapolations of mechanisms of action of calcium antagonists derived from one vessel cannot be readily extrapolated to other vessels. Our results even demonstrate that in many instances differences between various target vessels are more important than differences between species, as different as cat and rabbit, or calcium antagonists, as different as darodipine and verapamil (this does not apply to the effects on the myocardium).

It is interesting to note and perhaps surprising that calcium antagonists as different chemically as darodipine and verapamil, which have very different cardiac effects, show such similar profiles of activity in situations involving vasodilatation in supposedly autoregulated tissues, as well as in situations where vessels are activated by highly diverse vasoconstrictor agents such as A II, vasopressin or even ouabain (Hof & Hof, 1985).

In conclusion we have to modify the hypothesis stated in the Introduction that had been formulated on the basis of more limited data (Hof, 1984). Calcium antagonists appear to display two typical patterns of activity, both with minor variations. The vasodilator pattern consists of dilatation of the vessels of the heart, brain and, to a degree varying with the agents, skeletal muscle (Hof *et al.*, 1982a; Hof, 1983). The antivasoconstrictor effects occur in some but not all of the vessels constricted by the constrictor agent. Typically, constrictor effects occurring in the spleen, skin and arterio-venous shunts are not antagonized. This pattern appears to depend on the constrictor agent used, inasmuch as such agents constrict different vascular beds.

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