Effects of anipamil on cardiovascular status and regional blood flow in anaesthetized rats

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1 Anipamil, a long-acting analogue of verapamil, was tested at 1, 2.5 and 5 mg kg⁻¹ i.v. for its effects on cardiovascular status and blood flow in different organs and vascular beds in rats anaesthetized with pentobarbitone.

2 Blood flow was determined by microsphere (⁵⁷Co or ¹¹³Sn) injection before, and 1 h after, administration of anipamil at the above doses.

3 Anipamil produced a dose-dependent fall in blood pressure and heart rate. This was associated with a fall in peripheral resistance. At the highest dose there was, in addition, evidence of myocardial depression.

4 Both blood flow and conductance (flow corrected for blood pressure) were notably increased by anipamil in heart, liver and skeletal muscle beds, but were decreased, especially at the high doses, in kidneys, intestine and spleen.

5 The profile of the actions of anipamil on haemodynamic performance and flow was consistent with its proposed calcium antagonist actions. This profile was similar to that of verapamil.

Introduction

Anipamil is a verapamil analogue with a long duration of action (Raschack, 1984). It has been shown to be active against arrhythmias induced in conscious rats by occlusion of a coronary artery (Curtis *et al.*, 1986). Furthermore, it has anti-ischaemic actions in a variety of preparations (Gries & Raschack, 1984).

In *in vivo* experiments, anipamil lowered blood pressure and caused bradycardia (Curtis *et al.*, 1986). Dillon & Nayler (1988) recently found that anipamil binds to the same membrane sites as known calcium antagonists. In view of the above, it was considered of interest to ascertain the actions of anipamil in terms of its effects on blood pressure, heart rate, cardiac output and regional blood flow in order to determine whether or not its profile of cardiovascular actions was similar to those of other calcium antagonists. A preliminary report of parts of this study has been given previously (Walker *et al.*, 1988).

Methods

The effects of 1, 2.5 or 5 mg kg⁻¹ of anipamil i.v. on blood pressure, heart rate and regional blood flow were investigated in anaesthetized male Sprague-Dawley (University of British Columbia) rats weighing between 340–380 g. The procedures used have been described in detail elsewhere (Pang, 1983). Briefly, rats were anaesthetized with pentobarbitone (60 mg kg⁻¹, i.p.) and the iliac vein and arteries as well as the right carotid artery cannulated with PE-50 tubing. The carotid cannula was advanced into the left ventricle. Pressures were recorded with Statham pressure transducers connected to a Grass Polygraph (MP-7). Heart rate was electronically obtained by means of a Grass Tachograph (model 7P4F) while a Grass differentiator (model 7P20C) was used to process the ventricular pressure so as to obtain maximum *dp/dt*.

Suspensions of microspheres (15 µm diameter, New England Nuclear, Ma, U.S.A.) labelled either with ⁵⁷Co or ¹¹³Sn were injected either before, or 1 h after, administration of anipamil. Half of the animals

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received ^{57}Co microspheres before anipamil while ^{113}Sn was given 1 h after. In the rest of the animals this order of giving microspheres was reversed. No differences could be ascribed to the order in which labelled microspheres were given. Anipamil (Knoll AG, Ludwigshafen, W. Germany) was dissolved in warm (50°C) distilled water (acidified to pH 3.0 with HCl) and suitably diluted so as to give a maximum injection volume of 10 ml kg^{-1} . Similar volumes of acidified warmed water were used for control injections and all injections were given over 1 min.

The injection of the appropriate microsphere suspension (30,000–40,000 microspheres in $200\text{ }\mu\text{l}$) was made according to our usual procedure (Pang, 1983) with reference blood samples being withdrawn (Harvard infusion/withdrawal pump, Ma, U.S.A.) from the iliac arterial cannula at a rate of 0.35 ml min^{-1} for determination of cardiac output. After injection of the second set of microspheres the rat was killed and dissected into its component organs which were then weighed and their radioactive content determined as described previously (Pang, 1983). In the case of skin and muscle only representative samples were taken. The sample of skin was obtained from the dorsal and ventral areas and the muscle sample was taken from the chest, abdomen, limbs, diaphragm and back.

Data and statistical analysis

Blood flow in each of the organs was expressed either as total blood flow in the organ or tissue sample, or as conductance, i.e. flow divided by mean blood pressure. The latter normalisation procedure compensated for changes in blood pressure and allowed changes of resistance in different vessel beds to be assessed. Data were expressed either in absolute values in the pre- or post-drug periods, or as

changes (pre-drug minus post-drug). Drug effects could, therefore, be assessed relative either to the pre-drug period, or to vehicle-treated controls. Statistical tests were performed on group means by analysis of variance, for determining significant sources of variance, and by Duncan's multiple range test for difference between means using an SPSSS package on the UBC Amdahl mainframe computer. A *P* value of 0.05, or less, was taken as being statistically significant and all values are given as mean $\pm$ s.e.mean.

Results

General cardiovascular changes induced by anipamil

Figure 1 summarises the changes from the pre-drug values, shown in Table 1, that anipamil induced in blood pressure, cardiac output, dp/dt_{max} , heart rate, stroke volume and total peripheral resistance. Anipamil caused a dose-dependent fall in blood pressure (Figure 1a) which was associated with bradycardia (Figure 1b), especially at the highest dose. The fall in blood pressure was associated with an increase in cardiac output at the lower doses (Figure 1a) but with a decrease at the highest dose. The calculated peripheral resistance showed statistically significant vasodilatation occurred with the two lower doses (Figure 1c).

The maximum rate of rise of ventricular pressure (dp/dt_{max} in Figure 1b) fell with the highest doses of anipamil, whereas stroke volume always rose with treatment (Figure 1c). The overall haemodynamic response pattern was consistent with anipamil causing a decreased peripheral resistance at the two lower doses, whereas at the highest dose direct depressant effects on the heart also became pronounced.

Table 1 Control values (pre-drug) for the various treatment groups

Group	BP (mmHg)	CO (ml min^{-1})	Heart rate (beats min^{-1})	dp/dt_{max} (mmHg s^{-1})	SV (ml)	TPR (mmHg min ml^{-1})
Pre-vehicle	93 ± 3	90 ± 9	300 ± 30	7670 ± 360	0.31 ± 0.02	1.1 ± 0.1
Pre-Anipamil: (mg kg^{-1})						
Pre-1	91 ± 2	87 ± 8	330 ± 15	7480 ± 360	0.27 ± 0.03	1.1 ± 0.1
Pre-2.5	111 ± 5	90 ± 6	335 ± 10	9390 ± 870	0.27 ± 0.02	1.3 ± 0.1
Pre-5	112 ± 5	98 ± 5	360 ± 20	9530 ± 550	0.28 ± 0.02	1.2 ± 0.1

BP = blood pressure, CO = cardiac output, SV = stroke volume, TPR = total peripheral resistance. Each value is mean $\pm$ s.e.mean from 9 rats. Measurements were made 1 min before beginning drug treatment.

Figure 1 Effects of anipamil and vehicle on haemodynamic variables expressed as post-drug minus pre-drug control values for individual animals exposed to vehicle, 1.0, 2.5 or 5.0 mg kg⁻¹ i.v. anipamil. The effect on blood pressure (filled columns) and cardiac output (open columns) is shown in (a), heart rate (filled columns) and dp/dt_{max} (open columns) in (b) and stroke volume (filled columns) and total peripheral resistance (open columns) in (c). Each value is mean $\pm$ s.e.mean. Statistical significance is shown by (*) for comparison with pre-drug values and (†) for comparison with vehicle control.

The changes induced by anipamil have to be viewed against the changes which occurred with time in this preparation. Thus, for the vehicle-treated control animals, the time between the pre-drug and the post-drug sampling period (1 h) was associated with statistically significant increases in total peripheral resistance and blood pressure.

The effects of anipamil on blood flow were expressed as changes in blood flow (Figure 2) or as flow normalised for changes in blood pressure, i.e. conductance, in Figure 3. Tables 2 and 3 give the

Figure 2 Effects of anipamil on blood flow in organs or tissue samples expressed as post-drug minus pre-drug values. Vehicle-treated controls are shown as open columns and 1, 2.5 and 5 mg kg⁻¹ anipamil i.v. as closed, cross-hatched and hatched columns, respectively. All values are mean with s.e.mean shown by vertical bars; $n = 9$. Statistical comparisons are with pre-drug values (*) or vehicle-treated group (†).

pre-drug values from which changes in flow and conductance were calculated. In all figures statistical significance is indicated for two types of comparison. The first comparison was between the pre- and post-drug values, while the second was between the post-drug periods for treated versus control groups. In Figure 2 it can be seen, for the vehicle-treated control group, that flows in all organs in the post-drug administration periods tended to be lower than those in the corresponding pre-drug periods. While individual differences only reached statistical significance for caecum and testes, the statistical chance for flow in all post-drug periods being less than the corresponding pre-drug periods (as was the case in Figure 2), was 0.00024. This indicates that a general reduction in flow with time occurred in pentobarbitone-anaesthetized rats. A general decrease in flow was in keeping with the higher blood pressure and total peripheral resistance found in the post-drug period for control animals (Figure 1a). With anipamil treatment a different pattern of flow changes was observed in that increases in flow occurred in some organs and vessel beds. These increases reached statistical significance for heart, liver, muscle and skin. When compared with the

Figure 3 Effects of anipamil on conductance in organs or tissue samples expressed as post-drug minus pre-drug values. Vehicle-treated controls are shown as open columns and 1, 2.5 and 5 mg kg⁻¹ anipamil i.v. as closed, cross-hatched and hatched columns, respectively. All values are mean with s.e.mean shown by vertical bars; *n* = 9. Statistical comparisons are with pre-drug values (*) or the vehicle-treated group (†).

vehicle-treated control group, statistical significance was only achieved for heart and liver. In other organs the pattern was more reminiscent of the vehicle-treated animals since flow was generally less in the post-drug period. These decreases, as opposed to the increases noted above, were greater with

higher doses and were statistically greater than vehicle-treated controls for intestine, kidneys and spleen at the highest dose level of anipamil.

When flow was normalised for changes in blood pressure so as to give conductance values (Figure 3), a similar pattern to that described above was found. In vehicle-treated rats conductance was uniformly reduced in the post-drug period, indicating a general increase in resistance in all vessel beds. Most noticeable increases in conductance in the post-drug period were seen in the heart and liver since, for the three doses of anipamil, the increases in conductance in the two organs were statistically greater than control. In muscle, conductance in post-drug periods was always statistically greater than in the pre-drug periods. A similar tendency for a decrease in conductance in the post-drug period was particularly marked after the high dose of anipamil.

In summary, anipamil caused vasodilatation in muscle, heart and liver vascular beds but the high dose, particularly, accentuated the tendency for a decrease in flow with time which occurred in this anaesthetized rat preparation.

Discussion

Anipamil caused a dose-dependent reduction in blood pressure as a result of a reduced total peripheral resistance. In addition, at the highest dose, there was an added component of myocardial depression, as witnessed by attenuation of the maximum rate of rise in ventricular pressure and reduced heart rate. When administered at 1 and 5 mg kg⁻¹, i.v., in conscious rats anipamil also prolonged P-R intervals, as well as lowering blood pressure and heart rate (Walker, unpublished observation); further evidence

Table 2 Absolute blood flow to the various organs and tissue samples

Organ	Pre-vehicle	Pre-1 mg kg ⁻¹	Pre-2.5 mg kg ⁻¹	Pre-5 mg kg ⁻¹
Heart	5.0 ± 0.7	4.4 ± 0.2	5.4 ± 0.4	5.2 ± 0.2
Liver	1.9 ± 0.3	2.1 ± 0.4	1.8 ± 0.3	1.9 ± 0.5
Stomach	1.1 ± 0.2	1.0 ± 0.2	1.2 ± 0.1	1.3 ± 0.2
Intestine	13.1 ± 0.2	9.9 ± 1.3	12.1 ± 0.8	14.0 ± 1.2
Caecum	2.7 ± 0.5	1.8 ± 0.2	2.7 ± 0.3	2.8 ± 0.3
Colon	2.7 ± 0.5	2.2 ± 0.3	1.7 ± 0.3	2.3 ± 0.3
Kidney	18.0 ± 1.8	15.5 ± 1.3	20.0 ± 1.7	18.8 ± 1.6
Spleen	1.2 ± 0.2	1.2 ± 0.1	1.4 ± 0.1	1.9 ± 0.2
Muscle (40 g)	1.7 ± 0.3	1.8 ± 0.2	1.8 ± 0.2	1.9 ± 0.2
Skin (40 g)	3.7 ± 0.7	3.4 ± 0.4	3.5 ± 0.3	3.5 ± 0.5
Testes	1.5 ± 0.2	1.7 ± 0.3	1.5 ± 0.1	1.6 ± 0.2
Brain	1.6 ± 0.2	1.5 ± 0.2	1.6 ± 0.1	1.9 ± 0.2

Values (ml min⁻¹) are mean ± s.e.mean of determinations in 9 rats anaesthetized with pentobarbitone. All values are the resting values before injection of drug or control solution.

Table 3 Absolute conductance in the various organs and tissue samples

Organ	Pre-vehicle	Pre-1 mg kg ⁻¹	Pre-2.5 mg kg ⁻¹	Pre-5 mg kg ⁻¹
Heart	5.3 ± 0.7	4.9 ± 0.2	4.9 ± 0.2	4.7 ± 0.2
Liver	2.0 ± 0.3	2.2 ± 0.4	1.6 ± 0.2	1.6 ± 0.4
Stomach	1.3 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	1.2 ± 0.1
Intestine	14.2 ± 1.1	11.1 ± 1.5	11.0 ± 0.7	13.2 ± 2.0
Caecum	2.9 ± 0.4	2.0 ± 0.2	2.5 ± 0.3	2.6 ± 0.4
Colon	3.0 ± 0.3	2.5 ± 0.3	1.6 ± 0.2	2.1 ± 0.3
Kidney	19.4 ± 1.8	17.1 ± 1.3	18.3 ± 1.7	17.1 ± 1.5
Spleen	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.7 ± 0.2
Muscle (40 g)	1.8 ± 0.3	2.0 ± 0.2	1.6 ± 0.1	1.7 ± 0.2
Skin (40 g)	3.9 ± 0.7	3.7 ± 0.4	3.2 ± 0.4	3.2 ± 0.3
Testes	1.6 ± 0.2	1.9 ± 0.3	1.3 ± 0.1	1.5 ± 0.3
Brain	1.7 ± 0.1	1.7 ± 0.3	1.5 ± 0.1	1.8 ± 0.3

Values (ml min⁻¹ mmHg⁻¹ × 100) are mean ± s.e.mean of determinations in 9 rats anaesthetized with pentobarbitone. All values are the resting values before injection of drug or control solution.

of direct cardiac effects. Overall the haemodynamic effects of anipamil were, therefore, reminiscent of those due to high dose verapamil (Curtis *et al.*, 1986). A degree of vascular selectivity was exhibited by anipamil but overall the drug appears to have a small ratio of vascular to cardiac actions since the highest dose caused both severe negative inotropic and chronotropic effects, which led to a reduction of flow in most organs. The highest and most cardiac depressant dose of anipamil also reduced the overall decrease in total peripheral resistance, presumably because of marked activation of vasopressor systems in an attempt to maintain blood pressure in the presence of reduced cardiac output. In a recent study Dillon & Nayler (1988) reported that anipamil has a dose-dependent negative inotropic effect in spontaneously beating, or electrically-paced, rat isolated hearts. The same authors also examined the binding characteristics of anipamil to membranes from rat myocardium and found that anipamil binds to phenylalkylamine binding sites in a manner similar to verapamil and related calcium antagonists.

Although heart rate and maximum rate of rise in ventricular pressure were reduced by the administration of 2.5 mg kg⁻¹ anipamil, an increase in cardiac output was still observed. This increase was possibly the result of an increase in venous tone. It has previously been shown in our laboratory that the administration of verapamil, nifedipine or flunarizine cause dose-dependent increases in mean circulatory filling pressure (Waite *et al.*, 1988). Mean circulatory filling pressure is a measure of total body venous tone (Guyton *et al.*, 1973). An increase in venous return after verapamil (Taira *et al.*, 1980) or nifedipine (Ogilvie, 1985) has also been reported to occur in anaesthetised dogs. The suggestion that anipamil caused an increase in cardiac output by

increasing venous tone is supported by other reports to the effect that a number of calcium antagonists can increase cardiac output while reducing heart rate (Hof *et al.*, 1982; Flaim & Zelis, 1982).

Comparison of the flow and conductance changes revealed an interesting pattern of vasodilatation and vasoconstriction with different doses of anipamil. In vehicle-treated controls there was increasing vasoconstriction with time which was overcome to an extent by the lowest doses of anipamil. However, at the highest dose of anipamil, further constriction occurred. The low dose of anipamil (1 mg kg⁻¹) did not greatly change the flow in most vascular beds, except for the heart and liver where flow was markedly increased. Although the 2.5 mg kg⁻¹ dose of anipamil increased cardiac output, flow in a number of organs was reduced (particularly in caecum, spleen, skin, testes and brain) while it was increased in others (liver, heart and muscle). Such patterns suggest that anipamil selectively dilated blood vessels in coronary and skeletal muscle beds. The increase in hepatic arterial flow was possibly related to reduced portal venous flow. It has been shown that a decrease in portal venous flow leads to an increase in hepatic arterial flow such that total hepatic blood flow remains constant (Lautt, 1980).

Conductance values were calculated in order to normalise, or correct, blood flow for concomitant changes in blood pressure; such values more accurately reflect the true pattern of vasodilatation and vasoconstriction. In a comparison of conductance and flow, the pattern of vasodilatation in heart, liver and skeletal muscle was better revealed. In particular, calculation of conductance showed that the lower doses of anipamil, in fact, caused vasodilatation in the intestine, a finding which was not apparent from analysis of flow data. Generally, comparisons

between conductances in vehicle-treated and anipamil groups indicated that the lower doses of anipamil increased conductance in a number of organs to which flow had been reduced.

The overall haemodynamic and blood flow effects of anipamil had similarities and differences with other calcium antagonists. For example, verapamil caused a fall in blood pressure in anaesthetized cats which was dependent to some extent on cardiac depression (Hof, 1983). Verapamil also increased coronary blood flow but was noticeably more effective in causing vasodilatation in other vascular beds (Hof, 1983). In anaesthetized cats, PY 108-068, a dihydropyridine derivative, increased flow to heart,

muscle and the small intestine, as did nifedipine (Hof *et al.*, 1982).

In conclusion, it would appear that anipamil produces cardiovascular effects which are consistent with its proposed calcium antagonist actions. The haemodynamic and flow effects of anipamil are qualitatively similar to those produced by verapamil (unpublished observations and Curtis & Walker, 1986).

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