**British Journal of Pharmacology (1997) 120,** 1101–1113 

# Systemic, pulmonary and coronary haemodynamic actions of the novel dopamine receptor agonist in awake pigs at rest and during treadmill exercise Z1046

[1]Dirk J. Duncker, David B. Haitsma, Ingeborg E.J. van der Geest, Rene Stubenitsky, Jan R. van Meegen, *Arie J. Man in't Veld & Pieter D. Verdouw

Experimental Cardiology, Thoraxcenter and *Department of Internal Medicine I, Cardiovascular Research Institute COEUR, Erasmus University Rotterdam, Rotterdam, The Netherlands

**1** In view of the potential therapeutic application of specific dopamine receptor agonists in the treatment of hypertension and left ventricular dysfunction, we investigated the cardiovascular actions of the novel mixed $D_1/D_2$ dopamine receptor agonist Z1046 in awake pigs at rest and during treadmill exercise.

**2** Thirteen swine were chronically instrumented under sterile conditions for measurement of systemic, pulmonary, and coronary haemodynamics. Regional blood flows were determined with the radioactive microsphere technique.

**3** Z1046 (1, 10, 100 $\mu$g kg$^{-1}$, i.v.) produced dose-dependent reductions in central aortic blood pressure (up to $27\pm3\%$, $P\leqslant0.05$) in awake resting pigs which was accompanied by only minimal reflex activation of the sympathetic nervous system. The hypotensive response was principally the result of peripheral vasodilatation (system vascular resistance decreased up to $35\pm4\%$, $P\leqslant0.05$), which was located in the cerebral, coronary, renal, mesenteric, adrenal, splenic and skeletal muscular vascular beds (vascular resistance decreased up to 30–40% after the highest dose in these beds). Only in the cerebral and mesenteric bed was the vasodilatation sufficiently large to overcome the decrease in blood pressure and result in an increased blood flow; the vasodilatation in the coronary bed was most likely due to autoregulation as neither coronary blood flow nor myocardial oxygen demand were changed significantly by Z1046. The systemic vasodilatation that was caused by the highest i.v. dose (100 $\mu$g kg$^{-1}$) was accompanied by transient and minor increases in heart rate ($15\pm5\%$, $P\leqslant0.05$) and cardiac output ($15\pm5\%$, $P\leqslant0.05$) whereas after 10 $\mu$g kg$^{-1}$, i.v., a slight decrease in cardiac output also contributed to the hypotension. Z1046 had no effect on pulmonary vascular resistance.

**4** The systemic vasodilator responses to Z1046 (100 $\mu$g kg$^{-1}$, i.v.) were sustained during treadmill exercise (2–4 km h$^{-1}$ which produced heart rates of up to $233\pm10$ beats min$^{-1}$), but with increasing treadmill speed attenuation of the exercise-induced increase in heart rate ($-11\pm3\%$, $P\leqslant0.05$) and hence cardiac output ($-10\pm3\%$, $P\leqslant0.05$) (as stroke volume was not altered by Z1046) contributed significantly to a lower aortic blood pressure ($-20\pm3\%$, $P\leqslant0.05$). Z1046 had no effect on pulmonary vascular resistance during exercise.

**5** Oral administration of Z1046 (0.5, 1.5 mg kg$^{-1}$) produced a fall in central aortic blood pressure (up to $15\pm3\%$, $P\leqslant0.05$), which developed gradually during the first 90 min and lasted up to 4 h after administration, again with negligible changes in heart rate and $LVdP/dt_{max}$.

**6** Neither non-selective $\alpha$- and $\beta$-adrenoceptor blockade, nor selective $\alpha_2$-adrenoceptor blockade altered the vasodilator actions of Z1046, but non-selective $\alpha$- and $\beta$-adrenoceptor blockade abolished the cardiac responses to dopamine receptor stimulation, suggesting that its cardiac actions were principally caused by $D_2$-receptor-mediated inhibition of catecholamine release, whereas the vasodilator response was probably the result of vascular $D_1$-receptor stimulation.

**7** In conclusion, the novel dopamine receptor agonist Z1046 is an effective blood pressure lowering agent that elicits minimal reflex activation of the sympathetic nervous system in awake resting pigs. Systemic vasodilatation was not affected by combined $\alpha$- and $\beta$-adrenoceptor blockade, which is consistent with a predominantly $D_1$ receptor-dependent vasodilator mechanism. The hypotensive effect is maintained during treadmill exercise during which systemic vasodilatation and a lower cardiac output both contribute to the blood pressure lowering actions of Z1046. The cardiovascular profile of this orally active compound warrants further investigation of this class of drugs in experimental and clinical hypertension.

**Keywords:** Haemodynamics; dopamine receptor; systemic circulation; pulmonary circulation; organ blood flows; awake pig; exercise

## Introduction

In the early seventies it was shown that dopamine produced vasodilatation by a mechanism independent from $\beta$-adrenoceptors (Goldberg, 1972). Since then, two functionally separate dopamine (D-) receptor subtypes have been identified. $D_1$-receptors are located on arterial smooth muscle cells in the kidney and mesenteric vascular bed and in some species in coronary and cerebral vessels (Ueda *et al*., 1982; Kopia & Valocik, 1989; Zhao *et al*., 1990; Van Woerkens *et al*., 1991), where their stimulation causes vasorelaxation. $D_1$-receptors are

[1]Author for correspondence at: Experimental Cardiology, Thoraxcenter, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

also located on renal tubular cells and juxtaglomerular cells where they promote natriuresis and diuresis, respectively. $D_2$-receptors are situated on sympathetic nerve endings (inhibition of noradrenaline release), in the zona glomerulosa cells of the adrenal cortex (inhibition of angiotensin II-stimulated aldosterone release) and in sympathetic ganglia (inhibition of ganglionic transmission). Experimental and clinical evidence is accumulating that specific dopamine agonists may be of use in the treatment of hypertension and heart failure (Goldberg, 1972; Van Woerkens *et al*., 1991; 1992c; Rousseau *et al*., 1994). In particular, combined $D_1$-/$D_2$-receptor agonists could be of benefit in hypertension as they are likely to minimize baroreflex-mediated activation of the sympathetic nervous system.

Z1046 is an experimental dopamine receptor agonist which, compared to dopamine, exerts equipotent $D_1$-receptor and 30 times more potent $D_2$-receptor agonistic activity in rabbit splenic artery and isolated ear preparations, respectively (Pocchiari *et al*., 1994). Z1046 does not exhibit significant $\beta_1$-or $\beta_2$-agonistic properties in the guinea-pig atrium and trachea, respectively. However, the drug may have weak $\alpha_1$-antagonistic properties (rabbit aorta), while its $\alpha_2$-agonistic activity in the guinea-pig atrium may be similar to that of dopamine (Pocchiari *et al*., 1994). In the present study we investigated the systemic-, pulmonary- and coronary haemodynamic responses to i.v. and oral administration of Z1046 in awake resting pigs. In subsets of animals we also studied the responses to Z1046 after selective $\alpha_2$-adrenoceptor blockade or combined non-selective $\alpha$- and $\beta$-adrenoceptor blockade in order to determine whether adrenoceptor stimulation directly, or indirectly via presynaptic inhibition of catecholamine release, contributes to the haemodynamic effects of Z1046. Finally, in view of observations that $D_2$-receptor-mediated inhibition of catecholamine release may constitute a major component of the actions of Z1046 (Pocchiari *et al*., 1994; Marchini *et al*., 1994), we also studied its haemodynamic effects during sympathetic stimulation produced by graded treadmill exercise.

## Methods

Thirteen pigs were used in the present study. All experiments were performed in accordance with the 'Guiding Principles in the Care and Use of Laboratory Animals' as approved by the Council of the American Physiological Society and after approval of the Animal Care Committee of the Erasmus University Rotterdam. Adaptation of animals to the laboratory conditions started approximately 1 week before the day of surgery and continued until 1 week post-surgically.

### *Surgical procedures*

After an overnight fast, crossbred Landrace × Yorkshire pigs of either sex (20–28 kg) were sedated with ketamine (30 mg $kg^{-1}$, i.m.) anaesthetized with thiopental (20 mg $kg^{-1}$, i.v.) intubated and mechanically ventilated with a mixture of oxygen and nitrous oxide (1 : 2) to which 0.2–2% (v/v) isoflurane was added. Anaesthesia was maintained with midazolam (1 mg $kg^{-1}$ $h^{-1}$, i.v.) and fentanye (1 $\mu$g $kg^{-1}$ $h^{-1}$, i.v.). Under sterile conditions, the chest was opened via the fourth left intercostal space and an 8French (F) fluid-filled polyvinylchloride (PVC) catheter was

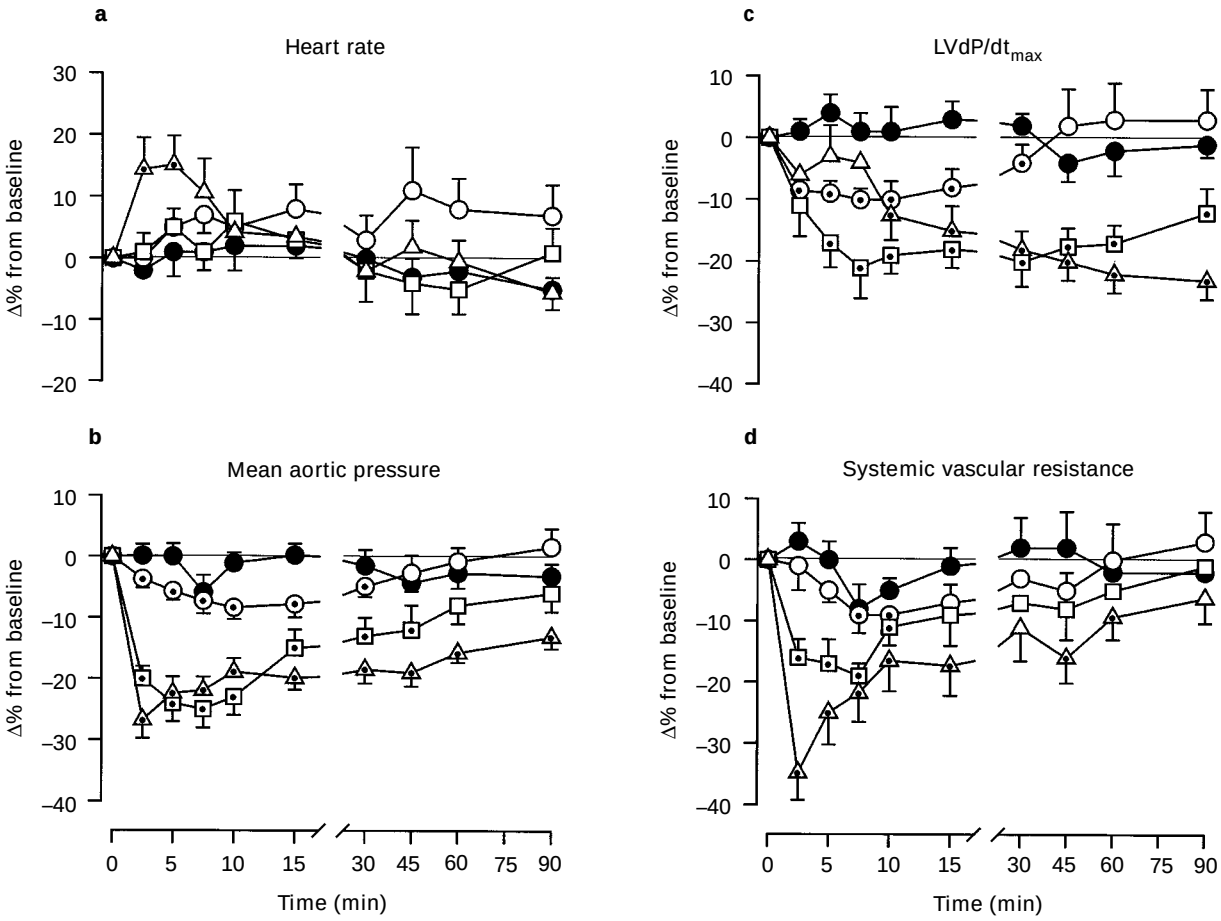


**Figure 1** Systemic haemodynamic effects of Z1046 i.v. in awake resting swine. The four treatment groups were saline (control $n=7$, ●), and three doses of Z1046: 1 $\mu$g $kg^{-1}$ ($n=9$, ○), 10 $\mu$g $kg^{-1}$ ($n=9$, □) and 100 $\mu$g $kg^{-1}$ ($n=10$, △). The variables measured were (a) heart rate, (b) mean aortic pressure, (c) $LVdP/dt_{max}$ and (d) systemic vascular resistance. Data are presented as mean and reflect % changes from pre-drug baseline; vertical lines show s.e.mean. Solid dot inside a symbol indicates a significant change ($P \leqslant 0.05$) from baseline (0 min).

**Table 1** Systemic and pulmonary haemodynamics after intravenous administration of Z1046 in awake resting pigs

| | *Z1046* ($\mu g\ kg^{-1}$) | *Baseline* | *2.5* | *5* | *7.5* | *10* | *15* | *30* | *45* | *60* | *90* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Time after administration (min)* | | | | | |
| CO | 0 | 4.2±0.3 | 4.1±0.3 | 4.2±0.3 | 4.3±0.3 | 4.4±0.3 | 4.2±0.3 | 4.1±0.3 | 4.0±0.3 | 4.2±0.3 | 4.2±0.3 |
| ($l\ min^{-1}$) | 1 | 3.7±0.2 | 3.6±0.3 | 3.6±0.2 | 3.7±0.3 | 3.7±0.2 | 3.6±0.2 | 3.6±0.2 | 3.7±0.3 | 3.7±0.3 | 3.7±0.2 |
| | 10 | 4.0±0.2 | 3.9±0.2 | 3.7±0.2 | 3.6±0.2* | 3.5±0.2* | 3.7±0.2 | 3.8±0.2 | 3.9±0.2 | 3.9±0.2 | 3.9±0.1 |
| | 100 | 4.2±0.3 | 4.8±0.3* | 4.4±0.3* | 4.2±0.2 | 4.2±0.3 | 4.2±0.2 | 3.9±0.2 | 4.1±0.2 | 3.9±0.1 | 3.9±0.1 |
| SAP | 0 | 122±4 | 122±5 | 122±6 | 115±4 | 120±5 | 122±5 | 119±5 | 117±3 | 117±4 | 117±3 |
| (mmHg) | 1 | 131±8 | 125±8* | 122±8* | 122±9* | 120±9* | 122±8* | 125±9 | 129±10 | 130±10 | 133±9 |
| | 10 | 124±4 | 106±4* | 101±4* | 101±4* | 103±3* | 110±3* | 111±4* | 111±5* | 115±4* | 117±5 |
| | 100 | 127±4 | 106±4* | 111±5* | 110±5* | 109±5* | 107±4* | 106±4* | 103±5* | 107±4* | 108±4* |
| MAP | 0 | 100±3 | 100±5 | 100±5 | 94±4 | 99±5 | 100±4 | 99±4 | 96±3 | 97±3 | 97±3 |
| (mmHg) | 1 | 106±6 | 102±6* | 100±6* | 99±7* | 97±6* | 98±6* | 101±6* | 104±7 | 106±7 | 108±6 |
| | 10 | 101±3 | 81±3* | 77±4* | 75±3* | 78±2* | 86±3* | 88±3* | 89±3* | 93±3 | 95±4 |
| | 100 | 104±3 | 76±3* | 81±3* | 81±3* | 84±3* | 83±3* | 85±3* | 84±4* | 88±3* | 90±3* |
| DAP | 0 | 77±6 | 76±7 | 78±6 | 70±4 | 75±6 | 78±5 | 77±5 | 72±5 | 74±4 | 74±4 |
| (mmHg) | 1 | 81±5 | 78±5 | 77±5* | 75±6* | 73±6* | 74±6* | 78±5 | 79±6 | 82±6 | 83±6 |
| | 10 | 78±4 | 59±3* | 56±4* | 55±3* | 60±2* | 65±3* | 67±3 | 67±3 | 70±4 | 73±5 |
| | 100 | 80±4 | 53±4* | 58±4* | 58±3* | 62±3* | 62±3* | 64±4* | 65±4* | 68±3* | 71±3* |
| SVR | 0 | 24.5±1.5 | 25.1±1.3 | 24.7±1.8 | 22.6±1.5 | 23.4±1.6 | 24.1±1.3 | 25.3±3.2 | 25.0±1.9 | 24.1±2.1 | 24.0±2.1 |
| ($mmHg\ min^{-1}$) | 1 | 28.5±2.2 | 28.4±3.0 | 27.2±2.5 | 25.7±2.0* | 25.8±2.1* | 26.4±2.0* | 27.7±2.4 | 27.1±2.5 | 28.4±2.9 | 29.2±2.5 |
| | 10 | 25.4±1.4 | 21.3±1.6* | 21.1±1.9* | 20.5±1.0* | 23.0±1.5* | 23.8±2.5 | 23.6±2.2 | 23.3±2.0 | 24.1±1.8 | 25.2±1.7 |
| | 100 | 25.4±1.7 | 16.3±1.4* | 18.8±1.5* | 19.4±0.9* | 21.0±1.8* | 20.5±1.0* | 22.1±1.3 | 20.9±0.9* | 22.6±1.0* | 23.5±1.4 |
| MPAP | 0 | 17±2 | 17±5 | 19±1 | 17±1 | 18±2 | 19±2 | 19±2 | 17±2 | 17±1 | 16±1 |
| (mmHg) | 1 | 18±2 | 18±1 | 18±1 | 18±2 | 18±2 | 18±1 | 19±2 | 20±3 | 18±2 | 20±2 |
| | 10 | 20±2 | 20±2 | 18±1 | 17±1 | 17±2 | 18±2 | 16±1 | 15±1 | 16±1 | 18±1 |
| | 100 | 17±1 | 22±1* | 21±2 | 19±2 | 17±1 | 17±1 | 16±2 | 17±2 | 17±2 | 17±1 |
| LAP | 0 | 4±1 | 4±1 | 5±2 | 4±2 | 5±2 | 4±2 | 6±2 | 4±2 | 5±2 | 3±2 |
| (mmHg) | 1 | 6±1 | 7±1 | 6±1 | 6±1 | 6±1 | 6±1 | 6±1 | 8±2 | 7±2 | 8±1 |
| | 10 | 5±1 | 4±1 | 3±1 | 3±1 | 5±1 | 5±2 | 4±1 | 4±1 | 4±1 | 5±1 |
| | 100 | 5±1 | 5±2 | 4±1 | 5±1 | 5±2 | 3±1 | 4±1 | 3±1 | 5±2 | 4±1 |
| PVR | 0 | 3.4±0.5 | 3.2±0.5 | 3.2±0.4 | 3.2±0.5 | 3.1±0.5 | 3.7±0.6 | 3.6±0.6 | 3.5±0.4 | 3.1±0.3 | 3.1±0.3 |
| ($mmHg\ min^{-1}$) | 1 | 3.2±0.5 | 3.3±0.6 | 3.6±0.7 | 3.6±0.6 | 3.5±0.6 | 3.4±0.5 | 3.6±0.5 | 3.4±0.5 | 3.4±0.4 | 3.5±0.5 |
| | 10 | 3.5±0.5 | 4.1±0.7 | 4.0±0.7 | 3.7±0.6 | 3.2±0.9 | 4.0±0.5 | 4.0±0.9 | 3.8±0.9 | 3.6±0.8 | 4.1±0.4 |
| | 100 | 2.8±0.2 | 3.4±0.1 | 3.1±0.2 | 2.9±0.2 | 3.0±0.1 | 3.1±0.1 | 3.0±0.4 | 3.2±0.4 | 3.1±0.4 | 3.4±0.5 |

CO = cardiac output; SAP, MAP, DAP = systolic, mean and diastolic aortic blood pressure, respectively; SVR = systemic vascular resistance; MPAP = mean pulmonary artery pressure; MLAP = mean left atrial pressure PVR = pulmonary vascular resistance. Data are mean ± s.e.mean; control group contained 7 animals; the 1, 10 and 100 $\mu g\ kg^{-1}$ groups contained 9, 9 and 10 pigs, respectively; *$P \leqslant 0.05$ vs baseline.

**Table 2** Left ventricular and coronary haemodynamics after intravenous administration of Z1046 in awake resting pigs

| | *Z1046* ($\mu g\ kg^{-1}$) | *Baseline* | *2.5* | *5* | *7.5* | *10* | *15* | *30* | *45* | *60* | *90* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Time after administration (min)* | | | | | |
| HR | 0 | 124±3 | 121±4 | 125±5 | 124±5 | 127±7 | 126±3 | 123±4 | 119±6 | 121±5 | 118±3 |
| (beats $min^{-1}$) | 1 | 115±7 | 116±8 | 122±8 | 124±9 | 122±9 | 124±8 | 118±8 | 126±8 | 124±8 | 123±8 |
| | 10 | 128±6 | 129±6 | 134±7 | 129±8 | 135±6 | 131±6 | 125±6 | 122±5 | 121±6 | 129±6 |
| | 100 | 122±4 | 139±7* | 141±7* | 135±8 | 127±7 | 127±6 | 120±7 | 124±6 | 121±4 | 115±4 |
| LVSP | 0 | 123±6 | 124±7 | 125±8 | 120±6 | 122±7 | 123±6 | 123±5 | 118±5 | 121±4 | 121±5 |
| (mmHg) | 1 | 130±11 | 124±10* | 122±10* | 121±11* | 120±10* | 123±10* | 124±11* | 124±12* | 128±11 | 130±10 |
| | 10 | 122±3 | 112±4* | 109±7* | 102±4* | 101±6* | 112±5* | 111±5* | 115±6 | 117±4 | 118±6 |
| | 100 | 126±4 | 109±3* | 115±5* | 109±4* | 109±3* | 108±4* | 108±4* | 109±5* | 108±4* | 110±4* |
| RPP | 0 | 14200±800 | 14200±1000 | 14900±1000 | 14200±900 | 14500±1200 | 14400±700 | 14200±800 | 13200±1100 | 13500±700 | 13300±600 |
| (mmHg beats $min^{-1}$) | 1 | 14100±400 | 13400±600 | 14100±600 | 14100±600 | 13700±900 | 14000±700 | 13400±600 | 13600±400 | 14300±900 | 14400±800 |
| | 10 | 14800±900 | 13600±800 | 13900±900 | 12600±1300* | 13300±1300 | 14100±1000 | 13100±700* | 13300±500* | 13200±1000 | 14400±900 |
| | 100 | 14900±600 | 14600±800 | 15700±1200 | 14400±1000 | 13600±900 | 13300±700* | 12600±800* | 13500±700* | 12900±400* | 12500±500* |
| LVEDP | 0 | 8±1 | 10±2 | 9±2 | 7±2 | 8±2 | 9±2 | 10±2 | 7±1 | 8±2 | 6±2 |
| (mmHg) | 1 | 8±2 | 8±2 | 7±2 | 6±2 | 7±2 | 7±2 | 6±3 | 7±2 | 8±2 | 11±2 |
| | 10 | 8±2 | 6±1 | 5±2 | 5±2 | 5±2 | 6±2 | 5±2 | 5±2 | 6±2 | 7±2 |
| | 100 | 8±1 | 8±2 | 8±2 | 5±2 | 6±2 | 6±2 | 7±1 | 7±2 | 6±2 | 7±1 |
| $LVdP/dt_{max}$ | 0 | 3360±180 | 3400±210 | 3490±210 | 3420±230 | 3410±220 | 3480±250 | 3430±190 | 3230±200 | 3290±190 | 3320±170 |
| (mmHg $s^{-1}$) | 1 | 3120±240 | 2850±180* | 2810±190* | 2800±180* | 2780±170* | 2850±200* | 3010±260* | 3140±190 | 3200±270 | 3160±170 |
| | 10 | 3030±150 | 2720±190 | 2490±140* | 2380±150* | 2450±160* | 2490±170* | 2410±150* | 2480±140* | 2500±120* | 2660±120* |
| | 100 | 3320±180 | 3030±170 | 3170±210 | 3140±200 | 2880±160* | 2790±160* | 2720±160* | 2710±190* | 2590±160* | 2560±140* |
| $LVdP/dt_{min}$ | 0 | −2730±190 | −2700±200 | −2700±230 | −2520±160 | −2670±220 | −2660±210 | −2570±220 | −2510±210 | −2550±210 | −2650±220 |
| (mmHg $s^{-1}$) | 1 | −2950±270 | −2800±230* | −2690±200* | −2640±220* | −2590±220* | −2710±260* | −2810±250 | −2820±180 | −2900±220 | −3030±360 |
| | 10 | −2620±180 | −2230±160* | −2010±190* | −1960±170* | −2060±160* | −2170±170* | −2220±180* | −2300±180* | −2330±170* | −2400±180 |
| | 100 | −2590±150 | −2030±140* | −2220±150* | −2120±170* | −2090±140* | −2130±150* | −2130±160* | −2120±170* | −2070±150* | −2190±150* |
| SV | 0 | 33.9±2.4 | 33.7±2.1 | 33.6±2.3 | 34.5±2.4 | 34.8±2.8 | 33.7±2.3 | 33.7±3.0 | 33.4±2.5 | 35.1±3.2 | 35.5±2.6 |
| (ml) | 1 | 30.8±2.7 | 30.1±2.7 | 28.9±2.8 | 29.2±3.1 | 28.8±2.7 | 27.7±2.1 | 30.6±2.6 | 29.1±2.4 | 28.3±2.0 | 29.0±2.2 |
| | 10 | 32.0±1.9 | 30.6±2.0 | 27.5±1.8* | 27.5±1.2* | 25.5±1.0* | 28.3±1.2* | 29.2±1.2 | 31.2±1.4 | 31.4±1.2 | 30.2±1.8 |
| | 100 | 35.1±2.2 | 34.1±1.7 | 31.8±2.0* | 31.9±2.0* | 32.5±1.7 | 33.0±1.5 | 33.0±1.6 | 33.2±1.9 | 32.2±1.5 | 34.1±1.5 |
| CBF | 0 | 33±4 | 34±4 | 35±4 | 33±4 | 34±5 | 34±4 | 33±4 | 33±4 | 32±4 | 33±4 |
| (ml $min^{-1}$) | 1 | 26±2 | 26±2 | 26±2 | 26±2 | 25±2 | 26±2 | 26±3 | 27±3 | 27±3 | 28±3 |
| | 10 | 28±3 | 30±4 | 27±3 | 28±4 | 27±3 | 28±3 | 28±3 | 28±4 | 28±3 | 30±3 |
| | 100 | 30±3 | 34±4 | 33±4 | 31±3 | 29±3 | 28±3 | 29±3 | 29±3 | 27±4 | 28±3 |
| CVR | 0 | 3.0±0.3 | 3.1±0.3 | 3.0±0.4 | 3.0±0.3 | 3.0±0.3 | 3.1±0.4 | 3.1±0.3 | 3.1±0.4 | 3.2±0.4 | 3.0±0.3 |
| (mmHg min $ml^{-1}$) | 1 | 4.1±0.3 | 4.0±0.3 | 4.0±0.3 | 3.9±0.3 | 3.9±0.3 | 3.8±0.3 | 4.0±0.4 | 4.0±0.4 | 4.0±0.4 | 4.0±0.4 |
| | 10 | 3.9±0.5 | 3.0±0.4* | 3.1±0.5* | 3.0±0.4* | 3.1±0.4* | 3.3±0.4 | 3.5±0.5 | 3.7±0.6 | 3.8±0.5 | 3.4±0.4 |
| | 100 | 3.7±0.5 | 2.5±0.5* | 2.7±0.5* | 2.6±0.3* | 3.2±0.6* | 3.1±0.5* | 3.1±0.4* | 3.1±0.4* | 3.5±0.6 | 3.4±0.5 |

HR = heart rate; LVSP = left ventricular peak systolic pressure; RPP = double product(HRxLVSP); LVEDP = LV end-diastolic pressure; $LVdP/dt_{max}$ = maximum rate of rise of LV pressure; $LVdP/dt_{min}$ = maximum rate of fall of LV pressure; SV = stroke volume: CBF = left anterior descending coronary artery blood flow; CVR = coronary vascular resistance. Data are mean ± s.e.mean; control group contained 7 animals; the 1, 10 and 100 $\mu\ kg^{-1}$ groups contained 9, 9 and 10 swine, respectively; *$P \leqslant 0.05$ vs baseline.

inserted into the aortic arch for measurement of blood pressure and secured with a purse string suture. After the pericardium was opened, an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta for measurement of aortic blood flow (cardiac output). A high fidelity pressure transducer (Konigsberg Instruments Inc., Pasadena, CA, U.S.A.) was inserted into the left ventricle via the apical dimple for recording of left ventricular pressure and its first derivative (LVdP/dt; obtained via electrical differentiation). An 8F PVC catheter was also inserted into the left ventricle for calibration of the Konigsberg transducer signal. Similar catheters were introduced into the left atrium for measurement of pressure and injection of radioactive microspheres to determine regional blood flows (see below) and in the pulmonary artery for measurement of pulmonary artery pressure and administration of drugs. A Doppler flow probe (2.0 or 2.5 mm in diameter, emitting frequency ($f_0$) = 20 MHz) was placed around the proximal part of the left anterior descending coronary artery to measure local coronary blood flow. Electrical wires and catheters were tunnelled subcutaneously to the back, the chest was closed and the animals were allowed to recover. All electrical wires and catheters were protected with a vest.

### *Post-surgical period*

During the first week after surgery animals received amoxicillin, 25 mg $kg^{-1}$, i.v. (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) and gentamycin, 5 mg $kg^{-1}$, i.v. (Alfasan, Woerden, The Netherlands) each day. Every day cathethers were flushed with physiological saline containing 2,000 iu $ml^{-1}$ heparin.

### *Regional myocardial blood flow measurements*

For the determination of regional blood flows, a batch of $1-2\times10^6$ carbonized plastic microspheres ($15\pm1$ $\mu$M (s.d.) in diameter) labelled with either $^{46}$Sc, $^{95}$Nb, $^{103}$Ru, $^{113}$Sn or $^{141}$Ce was injected into the left atrium. Starting 5 s before and continuing until 60 s after completion of the injection, a reference sample was withdrawn from the aorta at a rate of 10 ml $min^{-1}$. After all experimental protocols had been completed ($11\pm3$ days after injection of all five isotopes), animals were killed with an overdose of sodium pentobarbitone and various organs (heart, adrenals, liver, spleen, stomach, small intestine, brains and kidneys) and tissues (abdominal skin, various skeletal muscle groups) were excised, weighed and put into vials. The heart was divided into atria, right and left ventricle and fixed on formaldehyde (3.7% v/v). Two days later approximately 10 g of myocardium of the left ventricular free wall was divided into three layers of equal thickness: subepicardium, mesocardium and subendocardium. The radioactivity was counted and the amount of blood flow to the various tissue ($Q_{tis}$) calculated as:

$$Q_{tis} = (I_{tis}\ I_{art}{}^{-1}) \times Q_{art}$$

where $I_{tis}$ and $I_{art}$ are the radioactivity (c.p.m.) in a particular tissue and the arterial blood sample, respectively, while $Q_{art}$ is the rate of withdrawal of the blood sample. Blood flows were

**Table 3** Myocardial blood flow and vascular resistance responses to intravenous administration of Z1046 in awake resting pigs

| | *Z1046* ($\mu$g $kg^{-1}$) | *Baseline* | *5 min* | *30 min* |
|---|---|---|---|---|
| *Blood flows* (ml $min^{-1}$ 100 $g^{-1}$) | | | | |
| Right atrium | 10 | 30±3 | 38±5 | – |
| | 100 | 33±5 | 46±10 | 35±8 |
| Left atrium | 10 | 39±6 | 68±25 | – |
| | 100 | 47±7 | 81±27 | 60±15 |
| Right ventricle | 10 | 88±11 | 114±18 | – |
| | 100 | 108±14 | 125±25 | 104±16 |
| LVmean | 10 | 141±20 | 161±21 | – |
| | 100 | 161±18 | 173±31 | 152±25 |
| LVepi | 10 | 116±18 | 142±20 | – |
| | 100 | 137±17 | 150±27 | 132±23 |
| LVmeso | 10 | 155±22 | 174±22 | – |
| | 100 | 178±21 | 190±36 | 167±29 |
| LVendo | 10 | 167±21 | 178±23 | – |
| | 100 | 183±21 | 191±34 | 174±26 |
| endo/epi | 10 | 1.49±0.09 | 1.28±0.06 | – |
| | 100 | 1.35±0.08 | 1.28±0.10 | 1.35±0.05 |
| *Vascular resistances* (mmHg min $ml^{-1}$ 100g) | | | | |
| Right atrium | 10 | 3.48±0.39 | 2.62±0.54* | – |
| | 100 | 3.48±0.42 | 2.15±0.39* | 3.12±0.62 |
| Left atrium | 10 | 3.39±0.94 | 1.85±0.34* | – |
| | 100 | 2.32±0.29 | 1.31±0.24* | 1.83±0.43 |
| Right ventricle | 10 | 1.22±0.12 | 0.87±0.15* | – |
| | 100 | 1.01±0.10 | 0.71±0.09* | 0.90±0.12 |
| LVmean | 10 | 0.81±0.13 | 0.58±0.08* | – |
| | 100 | 0.65±0.05 | 0.50±0.06* | 0.61±0.07 |
| LVepi | 10 | 1.00±0.17 | 0.67±0.10* | – |
| | 100 | 0.78±0.07 | 0.57±0.06* | 0.71±0.09 |
| LVmeso | 10 | 0.74±0.13 | 0.53±0.07* | – |
| | 100 | 0.59±0.04 | 0.46±0.06* | 0.56±0.07 |
| LVendo | 10 | 0.67±0.11 | 0.52±0.07 | – |
| | 100 | 0.58±0.05 | 0.46±0.06* | 0.53±0.05 |

LV = left ventricle; mean = mean transmural, epi = subepicardium, meso = midmyocardium, endo = subendocardium. Data are mean ± s.e.mean; $n = 8$; *$P \leqslant 0.05$ vs baseline.

expressed as ml $min^{-1}$ 100 $g^{-1}$ tissue. Full details of the procedures and the calculation of flow data by this technique have been described previously (Van Woerkens *et al*., 1991; 1992b).

## *Experimental protocols*

Studies were performed between 1 and 5 weeks after surgery with animals either resting unrestrained and quietly in a cage or with animals exercising on a motor driven treadmill. The average weight of the thirteen animals increased from 28.5±1.4 kg to 35.4±1.0 kg over the four week period.

*Haemodyamic responses to increasing doses of intravenous Z1046 under resting conditions* Systemic, pulmonary and coronary haemodynamic responses to three doses of Z1046 were studied in resting pigs. After baseline haemodynamic measurements had been obtained, animals received i.v. injections, administered over a period of 1 min, of either 10 ml saline (vehicle, $n=7$), 1 $\mu$g $kg^{-1}$ ($n=9$), 10 $\mu$g $kg^{-1}$ ($n=9$) or 100 $\mu$g $kg^{-1}$ ($n=10$) of Z1046 dissolved in 10 ml saline. Following administration of saline or Z1046 the animals were studied for a 90 min period. Doses were administered in random order and each animal received only one dose daily. Consecutive experiments in the same animals were performed at a minimum interval of 48 h. Because of the limited number of radioisotopes that are available, regional blood flow measurements were made before and 5 min after administration of Z1046 in a dose of 10 $\mu$g $kg^{-1}$ and before and 5 and 30 min after administration of Z1046 in a dose of 100 $\mu$g $kg^{-1}$.

*Effects of intravenous Z1046 on the haemodynamic responses to treadmill exercise* In nine pigs we studied the effects of Z1046 (100 $\mu$g $kg^{-1}$, i.v.) during graded treadmill exercise. With swine resting on the treadmill (both lying and standing) measurements were made of systemic, pulmonary and coronary haemodynamics. Then, animals underwent a 3-stage exercise protocol (2, 3 and 4 km $h^{-1}$) with each level lasting 2–3 min in duration. Measurements were made during the last 30 s of each level of exercise when haemodynamics had reached a stable level. Following completion of the exercise protocol, animals were allowed to rest. Sixty minutes later, when all variables had returned to baseline, animals received either saline (10 ml, i.v.) or Z1046 (100 $\mu$g $kg^{-1}$, in 10 ml saline, i.v.). Five min later resting measurements were obtained and the exercise protocol was repeated.

*Haemodynamic responses to orally administered Z1046* In seven pigs we studied the haemodynamic responses to orally administered Z1046. After baseline measurements had been recorded swine received either placebo (empty gel caps) ($n=6$) or Z1046 in a dose of 0.5 mg $kg^{-1}$ ($n=7$) or 1.5 mg $kg^{-1}$ ($n=7$),

**Table 4** Regional blood flow and vascular resistance response to intravenous administration of Z1046 in awake resting pigs

| | *Z1046* ($\mu$g $kg^{-1}$) | *Baseline* | *5 min* | *30 min* |
|---|---|---|---|---|
| *Blood flows* (ml $min^{-1}$ 100 $g^{-1}$) | | | | |
| Brain | 10 | 81±4 | 92±10 | – |
| | 100 | 87±5 | 98±5* | 82±4 |
| Kidneys | 10 | 321±30 | 368±31 | – |
| | 100 | 339±26 | 381±26 | 350±27 |
| Adrenals | 10 | 114±8 | 134±11 | – |
| | 100 | 129±12 | 143±17 | 158±23 |
| Small intestine | 10 | 116±18 | 136±23 | – |
| | 100 | 120±18 | 147±27* | 95±13 |
| Stomach | 10 | 76±14 | 85±17 | – |
| | 100 | 85±18 | 67±11 | 56±6 |
| Liver | 10 | 23±6 | 19±10 | – |
| | 100 | 20±7 | 13±5 | 21±10 |
| Spleen | 10 | 155±25 | 162±25 | – |
| | 100 | 160±22 | 202±26 | 171±19 |
| Skeletal muscle | 10 | 6.7±1.3 | 9.8±2.3 | – |
| | 100 | 9.7±2.5 | 12.1±1.9 | 6.7±1.5 |
| Skin | 10 | 7.2±1.4 | 7.1±0.8 | – |
| | 100 | 7.2±0.9 | 8.6±1.5 | 7.5±1.1 |
| *Vascular resistances* (mmHg min $ml^{-1}$ 100 g) | | | | |
| Brain | 10 | 1.29±0.09 | 0.94±0.09* | – |
| | 100 | 1.24±0.08 | 0.81±0.06* | 1.03±0.07 |
| Kidneys | 10 | 0.35±0.04 | 0.23±0.03* | – |
| | 100 | 0.32±0.03 | 0.21±0.02* | 0.25±0.03* |
| Adrenals | 10 | 0.93±0.07 | 0.63±0.06* | – |
| | 100 | 0.86±0.08 | 0.61±0.10* | 0.64±0.14* |
| Small intestine | 10 | 1.06±0.16 | 0.76±0.15 | – |
| | 100 | 1.07±0.17 | 0.67±0.12* | 1.01±0.16 |
| Stomach | 10 | 1.84±0.40 | 1.27±0.27 | – |
| | 100 | 1.68±0.30 | 1.43±0.28 | 1.67±0.26 |
| Liver | 10 | 9.2±3.2 | 18.3±5.3 | – |
| | 100 | 10.8±2.7 | 10.5±2.2 | 11.5±2.8 |
| Spleen | 10 | 0.83±0.16 | 0.62±0.13* | – |
| | 100 | 0.82±0.17 | 0.46±0.10* | 0.53±0.07 |
| Skeletal muscle | 10 | 18.6±3.4 | 11.6±2.4* | – |
| | 100 | 15.5±3.8 | 7.2±1.1* | 15.0±2.5 |
| Skin | 10 | 18.5±3.3 | 12.5±1.6 | – |
| | 100 | 15.9±1.4 | 19.1±10.4 | 12.5±1.5 |

Data are mean±s.e.mean; $n=9$; *$P\leqslant 0.05$ vs baseline.

p.o., and were studied over a four hour period. Protocols were performed in random order at a minimum interval of 48 h.

*Modulation of adrenergic activity by Z1046* In three resting pigs we studied the effects of Z1046 (10 and 100 $\mu$g kg$^{-1}$, i.v.) in the presence of combined $\alpha$- and $\beta$-adrenoceptor blockade, 1.0 mg kg$^{-1}$ of the non-selective $\alpha$-adrenoceptor blocker phentolamine was administered followed by 1.0 mg kg$^{-1}$ h$^{-1}$, i.v. (Verdouw *et al*., 1984) and 0.5 mg kg$^{-1}$ of the non-selective $\beta$-adrenoceptor blocker propranolol followed by 0.5 mg kg$^{-1}$ h$^{-1}$, i.v. (Duncker *et al*., 1988). Ten minutes after administration of the blockers, when haemodynamics had reached a new steady state, Z1046 was administered in a dose of either 10 or (on a different day) 100 $\mu$g kg$^{-1}$, i.v., and haemodynamic measurements were made during the following 90 min.

In seven resting pigs we studied the effects of Z1046 (100 $\mu$g kg$^{-1}$, i.v.) in the presence of the $\alpha_2$-adrenoceptor antagonist yohimbine. Yohimbine was administered in a dose of 30 $\mu$g kg$^{-1}$, i.v., followed by a continuous infusion of 3 $\mu$g kg$^{-1}$ min$^{-1}$, i.v. After ten minutes, when haemodynamics had reached a steady state, Z1046 was administered and haemodynamic variables were recorded during the following 90 min.

*Haemodynamic responses to repeated administration of an identical dose of intravenous Z1046* On a different day we studied the systemic, pulmonary and coronary haemodynamic responses to two consecutive doses of Z1046 10 $\mu$g kg$^{-1}$, i.v., in 7 resting swine. The two doses of Z1046 were administered in 10 ml saline over 1 min separated by a 90 min interval.

## *Data acquisition and analysis*

Data were recorded and digitized with an eight channel data-acquisition programme ATCODAS (Dataq Instruments, Inc., Akron, Ohio, U.S.A.) and stored on a computer for later post-acquisition analysis with a programme written in MatLab (The Mathworks Inc., Mass, U.S.A.). Coronary blood flow was computed from the Doppler shift by use of the equation $Q = 1.25 \times \Delta f \times d^2$, where Q is the coronary blood flow (ml min$^{-1}$), $\Delta$f is the Doppler shift (KHz), d is the internal diameter of the coronary artery (mm) within the flow probe (Ishida *et al*., 1983). The factor 1.25 is a constant derived from the speed of sound in tissue ($C = 1.5 \times 10^5$ cm$^{-1}$), the frequency of the omitted sound beam ($f_0 = 20$ mHz), the cosine of the angle at which the sound beam is emitted (45°), and unit conversion factors: $(C \times 0.75\pi)/(2f_0 \times \cos\ 45°)$. Since in chronically instrumented animals the flow probe is tightly adherent to the coronary artery, the internal diameter of the flow probe is equal to the external diameter of the artery. To obtain the inner diameter of the coronary artery we subtracted the arterial wall thickness which is approximately 10% of the external diameter of the coronary artery. In this way any error in computation of the coronary internal diameter would affect control and intervention conditions equally. Systemic vascular resistance was calculated as the ratio of mean aortic pressure and cardiac output, pulmonary vascular resistance as the ratio of (mean pulmonary artery pressure − mean left atrial pressure) and cardiac output and regional vascular resistances as the ratio of mean aortic pressure and local blood flows. Statistical analysis was performed by analysis of variance for repeated measures. When a significant effect was observed *post-hoc* testing was done with Wilcoxon signed rank or paired *t* test. A *P* value of less than or equal to 0.05 was considered statistically significant (two-tailed). All data are presented as mean ± s.e.mean.

## *Drugs*

Z1046 ((**S**)-6-[[6-[[2-(2-methoxyphenoxy)ethyl]amino]hexyl]-propylamino]-5,6,7,8-tetrahydro-1,2-naphtalenediol dihydrochloride (courtesy of Dr F. Marchini, Zambon Group S.p.A., Italy), yohimbine and propranolol were dissolved in warm saline (30°C) to produce concentrations of 3 $\mu$g kg$^{-1}$ ml$^{-1}$ (yohimbine) and 0.05 mg kg$^{-1}$ ml$^{-1}$ (pro-

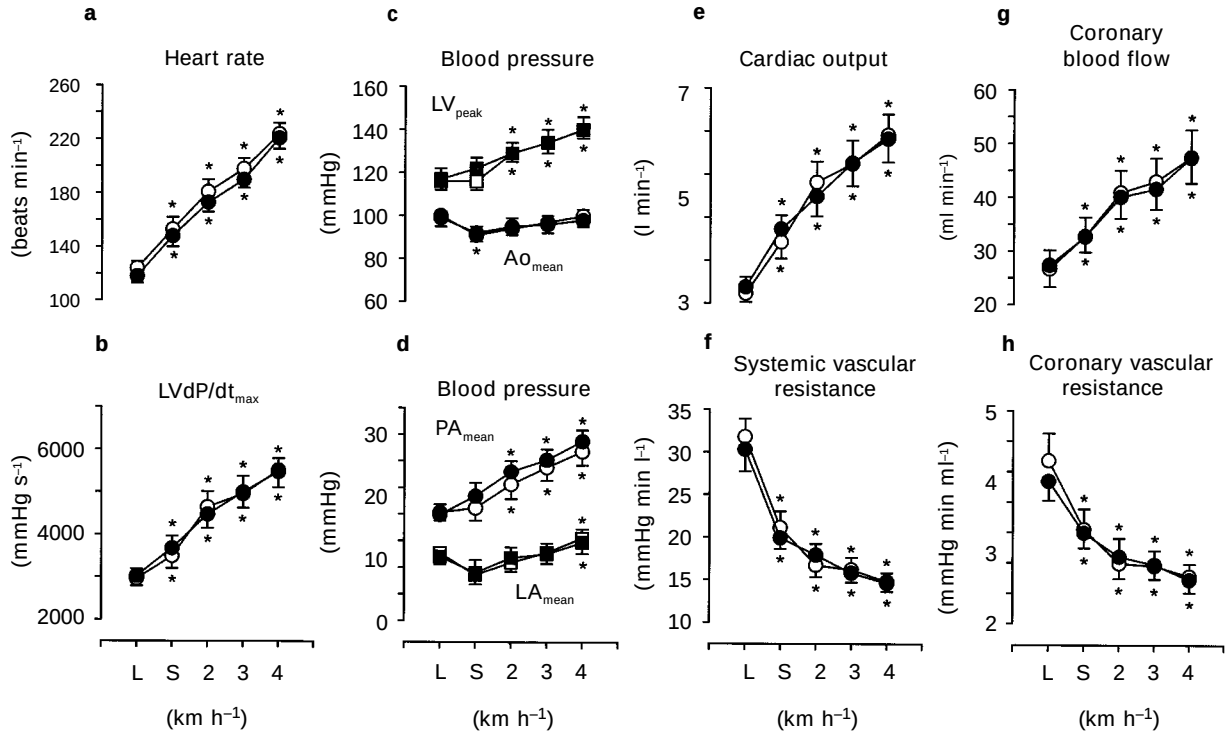


**Figure 2** Systemic haemodynamic variables during two consecutive periods of graded treadmill exercise in 9 pigs. (c) $LV_{peak}$ = left ventricular peak systolic pressure (■, □), $Ao_{mean}$ = mean aortic pressure (●, ○); (d) $PA_{mean}$ = mean pulmonary artery pressure (●, ○), $LA_{mean}$ = mean left atrial pressure (■, □). L = lying down, S = standing. Animals were studied during control conditions (open symbols) and following administration of saline (10 ml, solid symbols). Data are presented as mean; vertical lines show s.e.mean. $^*P \leqslant 0.05$ versus lying down; $^\bullet P \leqslant 0.05$ saline vs control.

pranolol), respectively. Phentolamine was dissolved in water containing glucose (35 mg $ml^{-1}$) and further diluted in saline to produce a final concentration of 0.1 mg $kg^{-1}$ $ml^{-1}$. Fresh drug solutions were prepared on the day of each experiment.

## Results

### *Haemodynamic responses to increasing doses of intravenous Z1046 in awake resting pigs*

*Saline* Administration of saline did not lead to significant changes in any of the systemic, pulmonary or coronary haemodynamic variables, demonstrating good cardiovascular stability over the 90 min observation period (Figure 1, Tables 1 and 2).

### *Z1046*

*Systemic and pulmonary haemodynamics* Z1046 in doses of 1, 10 and 100 $\mu g\ kg^{-1}$ produced dose-dependent decreases in mean aortic blood pressure which reach their peak values of $8\pm2\%$, $25\pm3\%$ and $27\pm3\%$, respectively, within the first 10 min after administration (Figure 1; Table 1). Thereafter, aortic pressure gradually recovered over the remainder of the 90 min period; the recovery was complete after 1 $\mu g\ kg^{-1}$ and 10 $\mu g\ kg^{-1}$ but not after 100 $\mu g\ kg^{-1}$. Similar decreases were observed in systolic and diastolic aortic pressure, so that arterial pulse pressure did not change. The Z1046-induced fall in blood pressure was caused by systemic vasodilatation as cardiac output (and its two determinants heart rate and stroke volume) was virtually unchanged. Only after administration of 10 $\mu g\ kg^{-1}$ did cardiac output and stroke volume decrease slightly, whereas after 100 $\mu g\ kg^{-1}$, cardiac output and heart rate transiently increased, probably due to reflex-mediated sympathetic activation (see below). Mean pulmonary artery and left atrial pressures were not affected except for a transient increase in pulmonary pressure during the first 2.5 min following administration of 100 $\mu g\ kg^{-1}$, which paralleled the increase in cardiac output. Consequently, pulmonary vascular resistance was not significantly altered by any dose of Z1046 (Tables 1 and 2). $LVdP/dt_{max}$ and $LVdP/dt_{min}$ decreased dose-dependently by up to $23\pm3$ and $21\pm3\%$, respectively, after 100 $\mu g\ kg^{-1}$ (both $P\leqslant0.05$) which may in part have been related to the decreases in diastolic aortic pressure and left ventricular systolic pressure, respectively.

*Coronary and myocardial haemodynamics* Coronary artery blood flow measured with the Doppler flow probe was not affected by any of the doses of Z1046, although coronary flow tended to increase during the first 7.5 min after administration of the highest dose, and tended to decrease thereafter, in parallel with the changes in myocardial oxygen demand, reflected by heart rate or the 'double product' (heart rate $\times$ left ventricular systolic blood pressure) (Table 2). Consequently, the decreases in computed coronary vascular resistance that occurred with the highest two doses were the result of autoregulation to compensate for the decrease in mean aortic blood pressure. Z1046 had no effect on the distribution of myocardial blood flow across the left ventricular wall, measured with radioactive microspheres, 5 min after administration of 10 $\mu g\ kg^{-1}$ or 5 and 30 min after administration of 100 $\mu g\ kg^{-1}$ (Table 3).

*Distribution of regional blood flows and vascular resistances* Z1046 in doses of 10 and 100 $\mu g\ kg^{-1}$, i.v., produced vasodilatation in the brain (up to $35\pm4\%$ decrease in vascular resistance 5 min after administration of 100 $\mu g\ kg^{-1}$), small intestine ($40\pm7\%$), kidneys ($30\pm6\%$), adrenals ($31\pm6\%$), spleen ($33\pm7\%$) and skeletal muscle ($43\pm14\%$) (Table 4). Only in the brain and small intestine was the vasodilatation sufficiently large to overcome the decrease in aortic blood pressure so that blood flow to these organs increased. Neither dose of Z1046 produced significant changes in vascular resistance in the stomach, liver or skin.

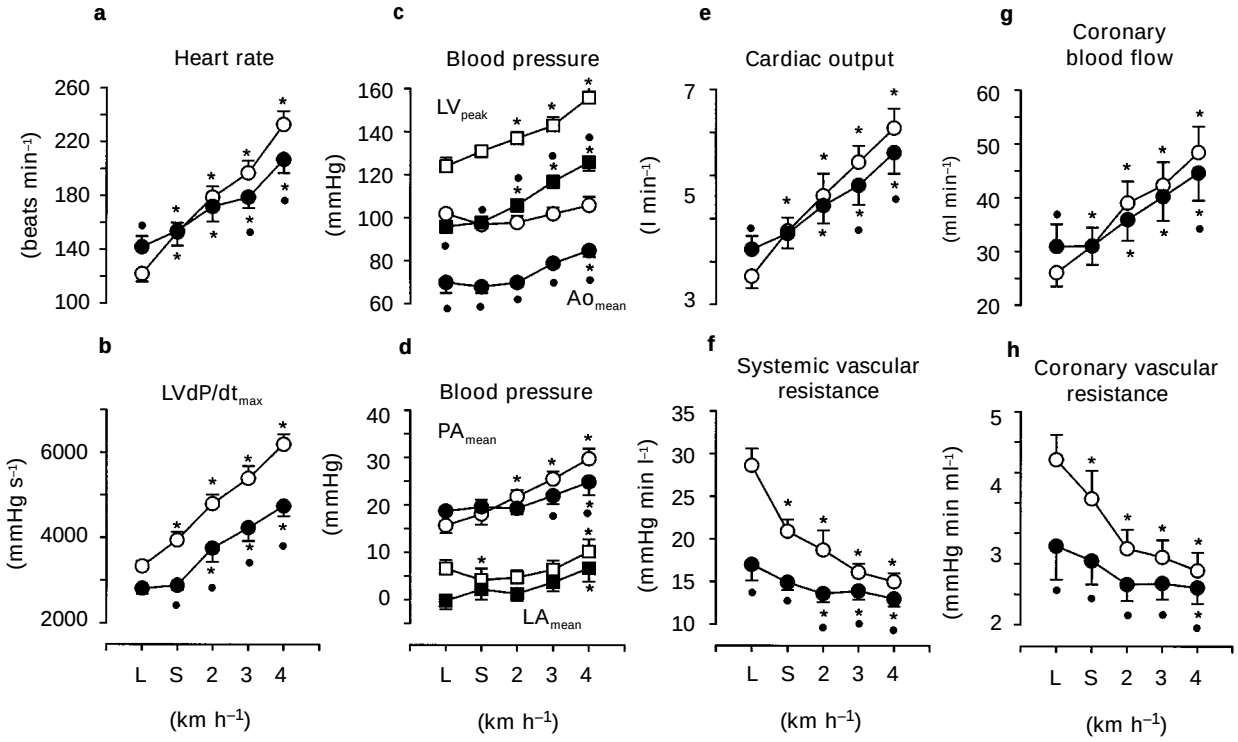


**Figure 3** Systemic haemodynamic effects of Z1046 100 $\mu g\ kg^{-1}$, i.v., in 9 pigs during graded treadmill exercise. (c) $LV_{peak}$ = left ventricular peak systolic pressure (■, □), Ao = mean aortic pressure (●, ○); (d) $PA_{mean}$ = mean pulmonary artery pressure (●, ○), $LA_{mean}$ = mean left atrial pressure (■, □); L = lying down, S = standing. Animals were studied during control conditions (open symbols) and in the presence of Z1046 (solid symbols). Data are presented as mean and vertical lines show s.e.mean. $^{*}P\leqslant0.05$ versus lying down; $^{\bullet}P\leqslant0.05$ Z1046 vs control.

*Effects of intravenous Z1046 on the responses to treadmill exercise*

*Saline* Exercise produced increases in heart rate from $124\pm5$ beats $min^{-1}$ at rest to $224\pm8$ beats $min^{-1}$ at the highest level of exercise (4 km $h^{-1}$), left ventricular systolic pressure from $116\pm4$ to $140\pm4$ mmHg, $LVdP/dt_{max}$ from $2960\pm170$ to $5510\pm280$ mmHg $s^{-1}$ and cardiac output from $3.2\pm0.2$ to $6.9\pm0.5$ l $min^{-1}$ (Figure 2). While mean aortic pressure increased only slightly from $91\pm3$ (quietly standing) to $100\pm3$ mmHg at the highest level of exercise, mean pulmonary artery pressure almost doubled from $15\pm1$ to $27\pm3$ mmHg. These responses reflect the intense systemic vasodilatation versus the unaltered pulmonary vascular resistance. Coronary blood flow increased from $27\pm3$ ml $min^{-1}$ at rest to $48\pm5$ ml $min^{-1}$ during the highest level of exercise. Two consecutive exercise periods separated by 60 min of rest, resulted in highly reproducible systemic, pulmonary and coronary haemodynamic responses.

*Z1046* During resting conditions, Z1046 in a dose of 100 $\mu$g $kg^{-1}$, i.v., produced a fall in aortic pressure which was entirely the result of systemic vasodilatation (35% decrease in systemic vascular resistance), as it was accompanied by transient increases in heart rate and cardiac output (both 15%). In contrast, Z1046 markedly attenuated the exercise-induced increases in cardiac output (Figure 3), so that both a decrease in cardiac output (10%) and vascular resistance (15%) contributed to the hypotensive effects of the compound during exercise. The blunted increase in cardiac output was principally the result of an attenuation of the exercise-induced tachycardia, as the response of stroke volume to exercise was not altered. Stroke volume was maintained compared to control exercise, despite a slight elevation in preload (LV end-diastolic pressure, not shown) and a reduction in afterload (LV systolic pressure), suggesting an attenuation by Z1046 of the increase in contractility produced by exercise, which is consistent with a $D_2$-receptor-mediated inhibition of catecholamine release from sympathetic nerve endings. This is also suggested by the attenuation of the exercise-induced increase in $LVdP/dt_{max}$, although underestimation of contractility may have occurred because of the lower aortic blood pressure. The effects of Z1046 on the exercise-induced coronary hyperaemia paralleled its effects on heart rate and the double product resulting in a slightly lower coronary blood flow at the highest level of exercise, compared to control. The exercise-induced increase in pulmonary artery pressure was blunted by Z1046 in a fashion parallel to its effect on cardiac output. Consequently, pulmonary artery resistance was not affected by Z1046 either at rest or during exercise.

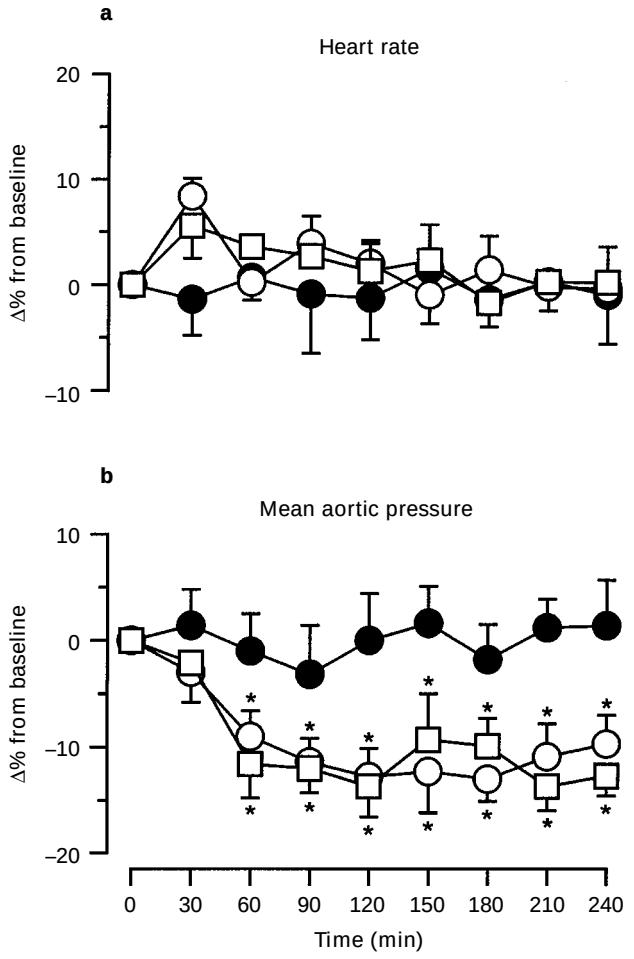


**Figure 4** Systemic haemodynamic effects (a) heart rate and (b) mean aortic pressure of Z1046, p.o., in awake resting pigs. The three treatment groups were placebo (control $n=6$, ●), and two doses of Z1046: 0.5 mg $kg^{-1}$ ($n=7$, ○) and 1.5 mg $kg^{-1}$ ($n=7$, □). Data (mean ± s.e.mean) are presented as percentage changes from pre-drug baseline. Baseline values of heart rate were $121\pm6$, $109\pm5$, and $118\pm6$ beats $min^{-1}$, in the placebo, 0.5 mg $kg^{-1}$ and 1.5 mg $kg^{-1}$ groups, respectively; baseline values of mean aortic blood pressure were $100\pm6$, $104\pm4$, and $101\pm4$ mmHg in the placebo, 0.5 mg $kg^{-1}$ and 1.5 mg $kg^{-1}$ groups, respectively. *$P\leqslant0.05$ vs baseline (0 min).

*Haemodynamic responses to oral Z1046*

*Placebo* Administration of placebo did not result in significant changes in heart rate (baseline value $121\pm6$ beats $min^{-1}$), mean aortic pressure (baseline value $100\pm6$ mmHg) (Figure 4), or any of the other systemic haemodynamic variables (not shown) over the 4 h observation period.

*Z1046* Oral administration of Z1046 in doses of 0.5 mg $kg^{-1}$ and 1.5 mg $kg^{-1}$ resulted in similar decreases in mean aortic

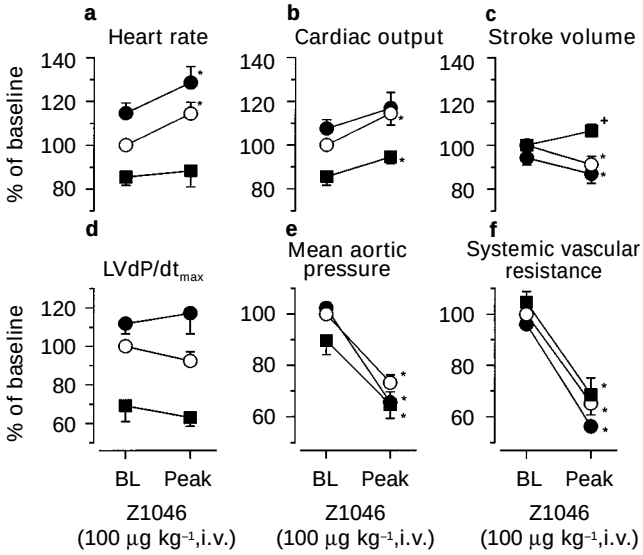


**Figure 5** Systemic haemodynamic peak effects of Z1046, i.v., in awake resting pigs. Z1046 was administered in a dose of 100 $\mu$g $kg^{-1}$ under control conditions ($n=10$, ○), in the presence of selective $\alpha_2$-adrenoceptor blockade ($n=7$, ●), and in the presence of combined $\alpha$- and $\beta$-adrenoceptor blockade ($n=3$, ■). Data are presented as mean % of predrug (i.e. pre-adrenoceptor blockade) baseline; vertical lines show s.e.mean. Baseline (BL) values for Z1046, 100 $\mu$g $kg^{-1}$, i.v., under control conditions (○) are presented in Table 1. Respective baseline values for the selective $\alpha_2$-adrenoceptor blockade group and the combined $\alpha$- and $\beta$-adrenoceptor blockade group were: (a) heart rate, $124\pm8$ and $132\pm3$ beats $min^{-1}$; (b) cardiac output, $4.2\pm0.3$ and $3.9\pm0.5$ l $min^{-1}$; (c) stroke volume, $34\pm3$ and $30\pm5$ ml; (d) $LVdP/dt_{max}$, $3320\pm160$ and $3370\pm210$ mmHg $s^{-1}$; (e) mean aortic pressure, $102\pm4$ and $88\pm5$ mmHg; (f) systemic vascular resistance, $25\pm2$ and $23\pm3$ mmHg $min\,l^{-1}$. *$P\leqslant0.05$ vs baseline, $^{+}P\leqslant0.05$ vs change in the non-blocked animals.

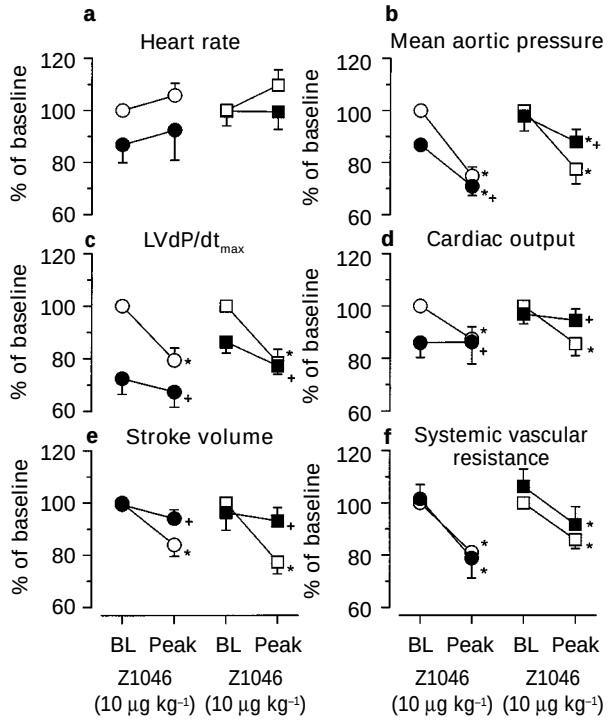


**Figure 6** Systemic haemodynamic peak effects of Z1046, i.v., in awake resting pigs. Z1046 was administered in a dose of 10 $\mu$g kg$^{-1}$ under control conditions ($n=9$, ○) and in the presence of combined $\alpha$- and $\beta$-adrenoceptor blockade ($n=3$, ●) or during two consecutive administrations of 10 $\mu$g kg$^{-1}$ under control conditions ($n=7$). The second dose (■) was administered 90 min after the first dose (□). Data (mean±s.e.mean) are presented as percentage of predrug baseline (i.e. pre-adrenoceptor blockade or pre-first dose). Baseline values for Z1046, 10 $\mu$g kg$^{-1}$, i.v., under control conditions (○) are presented in Table 1. Respective baseline values for the combined $\alpha$- and $\beta$-adrenoceptor blockade group and the two consecutive 10 $\mu$g kg$^{-1}$ group were: (a) heart rate, 105±14 and 120±6 beats min$^{-1}$; (d) cardiac output, 3.2±0.6 and 3.9±0.5 l min$^{-1}$; (e) stroke volume, 31±5 and 34±2 ml; (c) LVdP/dt$_{max}$, 2700±90 and 3060±190 mmHg s$^{-1}$; (b) mean aortic pressure, 101±6 and 98±4 mmHg; (f) systemic vascular resistance, 33±5 and 23±3 mmHg min l$^{-1}$. *$P\leqslant0.05$ vs baseline, $^{+}P\leqslant0.05$ vs change in the non-blocked animals or vs change produced by first dose.

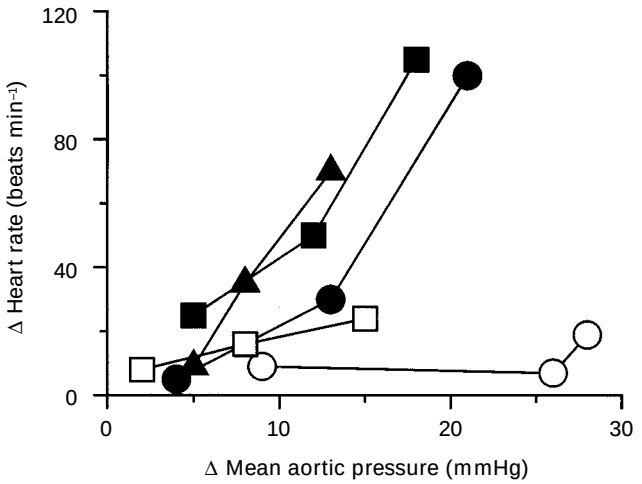


**Figure 7** Comparison of the reflex-tachycardia in response to systemic hypotension produced by Z1046 (○, present study), the dopamine receptor agonist epinine (□, Van Woerkens *et al*., 1992b), the calcium channel blocker nisoldipine (●, Duncker *et al*., 1987), and the $K^{+}_{ATP}$ channel activators nicorandil (■, Verdouw *et al*., 1987) and bimakalim (▲, Van Woerkens *et al*., 1992a) in awake resting pigs. Data are presented as absolute changes in heart rate vs absolute changes in aortic pressure. For the sake of clarity we have only shown mean data. Note that Z1046 lowered aortic blood pressure with minimal reflex-tachycardia.

blood pressure (baseline values 104±4 and 101±4 mmHg, respectively), which developed gradually over the first 90 min, reached a peak at 120 min (up to 15±2% and 15±3% after 0.5 and 1.5 mg kg$^{-1}$, respectively) and remained virtually unchanged during the remainder of the 4 h observation period (Figure 4). The decrease in mean aortic pressure was produced by a decrease in systemic vascular resistance (up to 11±3% and 14±4% after 0.5 and 1.5 mg kg$^{-1}$, respectively) whereas the slight decrease in cardiac output was not significant (not shown). LVdP/dt$_{max}$ decreased by up to 15% with either dose, while left ventricular end-diastolic pressure did not change (not shown). Neither 0.5 nor 1.5 mg kg$^{-1}$ had a significant effect on heart rate (baseline values 109±5 and 118±6 beats min$^{-1}$, respectively).

## *Modulation of adrenergic activity by Z1046*

*Non-selective α- and β-adrenoceptor blockade* Non-selective $\alpha$- and $\beta$-adrenoceptor blockade in three pigs resulted in decreases in heart rate and LVdP/dt$_{max}$ by approximately 20% and 35%, respectively, and a small increase in left ventricular end-diastolic pressure; mean aortic pressure fell by 15% due to the 18% decrease in cardiac output (Figure 5). Coronary blood flow decreased in parallel to the decrease in double product (not shown). Z1046 100 $\mu$g kg$^{-1}$, i.v., produced similar decreases in aortic pressure and systemic vascular resistance compared to animals with unblocked adrenoceptors, but with no change in heart rate. Thus, combined non-selective $\alpha$- and $\beta$-adrenoceptor blockade did not modify the vasodilator activity of Z1046, but prevented the mild reflex-tachycardia produced by the high dose of Z1046.

When Z1046 in a dose of 10 $\mu$g kg$^{-1}$, i.v., was administered in the presence of combined adrenoceptor blockade it produced considerably smaller (17 mmHg) decreases in aortic pressure compared to animals with unblocked adrenoceptors (26 mmHg, Figure 6). The decrease in pressure was produced solely by a decrease in systemic vascular resistance whereas the decreases in cardiac output and LVdP/dt$_{max}$, that was observed when adrenoceptors were unblocked, were absent in the presence of non-selective $\alpha$- and $\beta$-adrenoceptor blockade. These findings suggest that the cardiac effects of Z1046 but not the systemic vasodilator response were the result of inhibition of sympathetic activity.

*Selective $\alpha_2$-adrenoceptor blockade* Pretreatment with the selective $\alpha_2$-adrenoceptor antagonist yohimbine (30 $\mu$g kg$^{-1}$ and 3 mg kg$^{-1}$ min$^{-1}$, i.v.) resulted in slightly higher levels of heart rate, LVdP/dt$_{max}$ (Figure 5) and the double product (not shown), which was probably the result of increased noradrenaline release produced by presynaptic $\alpha_2$-adrenoceptor blockade. However, yohimbine did not alter the haemodynamic response to Z1046 (100 $\mu$g kg$^{-1}$, i.v.), indicating that Z1046 did not inhibit catecholamine release via presynaptic $\alpha_2$-adrenoceptor stimulation and that Z1046 was devoid of postsynaptic $\alpha_2$-adrenoceptor-mediated vasoconstrictor actions in awake swine.

## *Haemodynamic responses to repeated administration of an identical dose of intravenous Z1046*

Ninety minutes following administration of Z1046 (10 $\mu$g kg$^{-1}$, i.v.), at a time when all haemodynamic variables had returned to baseline except for LVdP/dt$_{max}$ which was still slightly (14±4%, $P\leqslant0.05$) below baseline levels, a second dose of 10 $\mu$g kg$^{-1}$ resulted in a markedly smaller decrease of aortic pressure (9±2 mmHg versus 23±6 mmHg, $P\leqslant0.05$). This attenuated hypotensive effect was not due to mitigation of the systemic vasodilator response but was the result of a maintained stroke volume and hence cardiac output following the second administration of 10 $\mu$g kg$^{-1}$ (Figure 6).

## Discussion

The most important findings of the present study were that (*i*) i.v. or oral administration of the non-selective dopamine receptor agonist Z1046 produced dose-dependent reductions in central aortic blood pressure in awake resting pigs which were accompanied by only minimal reflex activation of the sympathetic nervous system; (*ii*) the hypotensive response was principally the result of peripheral vasodilatation, in particular of the cerebral, coronary, renal, mesenteric, adrenal, splenic and skeletal muscular vascular beds; (*iii*) the vasodilatation that was caused by the highest i.v. dose (100 $\mu$g kg$^{-1}$) was accompanied by transient and minor, reflex-mediated, increases in heart rate and cardiac output, whereas after an i.v. dose of 10 $\mu$g kg$^{-1}$ a slight decrease in cardiac output also contributed to the hypotension; (*iv*) the systemic vasodilator response to dopamine receptor stimulation was sustained during treadmill exercise, but with increasing intensity of exercise, attenuation of the increase in cardiac output contributed significantly to the fall in pressure. (*v*) Non-selective $\alpha$- and $\beta$-adrenoceptor blockade did not alter the vasodilator actions of Z1046, but abolished the cardiac responses to dopamine receptor stimulation, suggesting that its cardiac actions were principally caused by $D_2$-receptor mediated inhibition of catecholamine release, whereas the vasodilator response was probably the result of vascular $D_1$-receptor stimulation.

### *Haemodynamic effects of D-receptor stimulation in awake resting swine*

Z1046 in doses of 1, 10 and 100 $\mu$g kg$^{-1}$, i.v., produced dose-dependent decreases in systemic vascular resistance of 10%, 20% and 35%, respectively. The response of cardiac output to Z1046 differed with each dose. Thus, while 1 $\mu$g kg$^{-1}$ had no effect, 10 $\mu$g kg$^{-1}$ produced a decrease and 100 $\mu$g kg$^{-1}$ produced a transient increase in cardiac output. The decrease in cardiac output produced by 10 $\mu$g kg$^{-1}$ was due to a decrease in stroke volume with no change in heart rate. A decrease in stroke volume in the face of a decrease in left ventricular systolic pressure indicates a negative inotropic effect, which is supported by the moderate decrease in $LVdP/dt_{max}$. Interpretation of changes in $LVdP/dt_{max}$ as changes in contractility in the presence of changes in aortic blood pressure is complicated by the dependency of $LVdP/dt_{max}$ on diastolic aortic pressure. Nonetheless, $LVdP/dt_{max}$ was still depressed 90 min after administration of 10 $\mu$g kg$^{-1}$ at a time when diastolic aortic pressure had returned to baseline levels. Furthermore, when 10 $\mu$g kg$^{-1}$ was administered after adrenoceptor blockade, no decrease in $LVdP/dt_{max}$ occurred despite a significant decrease in aortic pressure. In awake resting pigs, non-selective $\beta$-adrenoceptor blockade produced by propranolol results in a decrease in $LVdP/dt_{max}$ of 25% (Duncker *et al*., 1987; 1988), indicating that even under quiet resting conditions there is some degree of sympathetic activity. The observation that Z1046 decreased contractility in the present study is therefore consistent with inhibition of noradrenaline release from the sympathetic nerve endings produced by presynaptic $D_2$-like receptor stimulation. Z1046 in a dose of 100 $\mu$g kg$^{-1}$ produced a dramatic decrease in mean aortic pressure which was entirely due to a decrease in systemic vascular resistance. The minimal effect of the highest dose on cardiac output was somewhat surprising. If 10 $\mu$g kg$^{-1}$ produced a decrease in cardiac output via $D_2$-like receptor-mediated inhibition of sympathetic activity to the heart (which is suggested by the mild decreases in cardiac output and $LVdP/dt_{max}$), it would be expected that a higher dose would produce an even greater $D_2$-receptor-mediated depression of cardiac output. However, the greater peripheral vasodilatation, resulting in a slightly greater blood pressure reduction at 2.5 min after administration produced by the high dose may have resulted in reflex-mediated activation of sympathetic nervous system and withdrawal of cardiac vagal tone thereby counteracting the direct effects of $D_2$-like receptor stimulation on sympathetic activity. This hypothesis is supported by the observation that 100 $\mu$g kg$^{-1}$ resulted in transient ($<5$ min) increases in heart rate and cardiac output, that were absent when animals were pretreated with combined $\alpha$- and $\beta$-adrenoceptor blockade. It is important to note that in our awake swine model, Z1046 produced severe hypotension with considerably less reflex-tachycardia than produced by pure vasodilators such as the dihydropyridine calcium channel blockers (Duncker *et al*., 1988) or $K^+_{ATP}$ channel activators like nicorandil (Verdouw *et al*., 1987) and bimakalim (Van Woerkens *et al*., 1992a) that we have studied in the same model (Figure 7). The dopamine receptor agonist epinine, which also had some $\beta_2$-adrenoceptor agonistic properties in this model, produces slightly greater increases in heart rate at a given reduction in mean aortic blood pressure (Van Woerkens *et al*., 1992b).

The observation that the decrease in aortic blood pressure produced by Z1046 was blunted following repeated administration of the same dose (10 $\mu$g kg$^{-1}$, i.v.) deserves some comment. The smaller decrease in aortic blood pressure was not due to mitigation of the systemic vasodilator response but was the result of a maintained $LVdP/dt_{max}$, stroke volume and hence cardiac output following the second administration of 10 $\mu$g kg$^{-1}$. Although the baseline conditions were different, the response to Z1046 in the presence of $\alpha$- and $\beta$-adrenoceptor blockade resembled the second 10 $\mu$g kg$^{-1}$ administration of Z1046 (Figure 6). This could suggest that the altered responses to the second repeated administration of Z1046 (10 $\mu$g kg$^{-1}$) may have been the result of persistent inhibition of catecholamine release to the heart. The finding that 90 min after administration of the first dose all haemodynamic variables had returned to the baseline value with the exception of $LVdP/dt_{max}$ (which was still depressed) is also consistent with a persisting myocardial $D_2$-receptor stimulation.

Z1046 has previously been shown to exhibit weak $\alpha_1$-adrenoceptor antagonistic properties in rabbit aorta and $\alpha_2$-agonistic properties in guinea-pig atria (Pocchiari *et al*., 1994). In the present study, the systemic vasodilator response to Z1046 was not altered by combined $\alpha$- and $\beta$-adrenoceptor blockade, suggesting that vasodilatation to Z1046 does not involve $\alpha$- or $\beta$-adrenoceptors. However, balanced $\alpha_1$-antagonistic and $\alpha_2$-agonistic activities could have obscured an effect of non-selective $\alpha$-adrenoceptor blockade with phentolamine. That this is unlikely is suggested by the observation that the selective $\alpha_2$-adrenoceptor blocker yohimbine, had no effect on the systemic dilatation produced by Z1046. The vasodilatation produced by Z1046 was located in the brain, small intestine, kidneys, adrenals, spleen and skeletal muscle, with regional vascular resistances decreasing by 30–40%. Vasodilatation in the brain, kidneys, small intestine and adrenals was most probably the result of stimulation of $D_1$-like receptors which are abundant in these vascular beds. In support of this hypothesis, we have previously observed in the identical swine model that dopamine (in the absence or presence of non-selective $\alpha$- and $\beta$-adrenoceptor blockade) or the selective $D_1$-like receptor agonist fenoldopam, produced vasodilatation in these beds (Van Woerkens *et al*., 1991). Conversely, pretreatment with the selective $D_1$-like receptor antagonist SCH23390 abolished the renal vasodilatation produced by Z1046 administrated in a dose of 30 $\mu$g kg$^{-1}$, i.v. (Pradella *et al*., 1995).

We previously observed that non-selective $\alpha$- and $\beta$-adrenoceptor blockade abolished the vasodilator response to dopamine in the spleen while the vasodilatation in the kidneys and small intestine persisted (Van Woerkens *et al*., 1991), suggesting that the vasodilator response to Z1046 in the spleen could be due to $D_2$-like mediated withdrawal of $\alpha$-adrenergic tone. It is possible that the splenic $D_2$-like vasodilatation is species-specific for pigs, since vasodilatation produced by Z1046 in the rabbit isolated splenic artery is antagonized by the $D_1$-like receptor antagonist SCH23390 (Pocchiari *et al*., 1994). That the skeletal muscle vasodilatation is most probably due to $D_2$-like receptor stimulation is suggested by a previous study in anaesthetized dogs, in which Z1046 (30 $\mu$g kg$^{-1}$, i.v.) caused a 47% decrease in resistance of the femoral bed (which

represents mainly skeletal muscle resistance) that was blocked by the selective $D_2$-like receptor antagonist domperidone (Pradella *et al*., 1995). In contrast, in anaesthetized pigs we recently failed to observe vasodilatation in skeletal muscle tissue in response to Z1046 administered in doses of 10 and 100 $\mu g\ kg^{-1}$ (Duncker *et al*., unpublished observations). Similarly, we previously found that dopamine either in the absence or presence of $\alpha$- or $\beta$-adrenoceptor blockade failed to elicit a vasodilator response in skeletal muscle of anaesthetized pigs (Van Woerkens *et al*., 1991). In pentobarbitone anaesthetized dogs sympathetic activity is higher than in pentobarbitone anaesthetized pigs, due to pentobarbitone-induced vagal withdrawal which is prominent in dogs (Vatner, 1978). This would allow $D_2$-like receptor stimulation to produce vasodilatation via inhibition of $\alpha$-adrenoceptor-mediated constriction in dogs. In awake pigs sympathetic activity is higher than in anaesthetized animals (Van Woerkens *et al*., 1991; present study); this may be due to the inhibitory effects of barbiturates on sympathetic outflow (Roberts, 1980).

In the present study Z1046 had no effect on coronary artery blood flow or its distribution across the left ventricular myocardial wall. Since the double product was also minimally affected it is most likely that the decrease in coronary vascular resistance was principally the result of autoregulation. $D_1$-like receptor stimulation has been shown to produce coronary vasodilatation in anaesthetized dogs (Schuelke *et al*., 1971; Toda & Hatano, 1979; Kopia & Valocik, 1989) although one study in dogs was negative (Hieble *et al*., 1987). In the pig, we failed to observe $D_1$-like mediated coronary vasodilatation after intracoronary infusions of fenoldopam or i.v. infusions of dopamine in the presence of adrenoceptor blockade (Van Woerkens *et al*., 1991). Those findings suggest that the presence of $D_1$-like receptors in the coronary circulation could be species-dependent. In human isolated coronary arteries precontracted with noradrenaline, fenoldopam failed to elicit a vasodilator response in 6 out of 7 patients (Hughes & Sever, 1989), suggesting that human conduit coronary arteries do not possess a significant number of $D_1$-like receptors.

### *Haemodynamic effects of D-receptor stimulation during treadmill exercise*

In the present study we observed that Z1046 (100 $\mu g\ kg^{-1}$, i.v.) produced markedly different haemodynamic responses during treadmill exercise than during resting conditions. Thus, at rest the fall in aortic pressure was the result of systemic vasodilatation (35% decrease in systemic vascular resistance) despite a mild reflex tachycardia and increase in cardiac output, whereas during exercise hypotension was due to both a 15% decrease in vascular resistance and a 10% decrease in cardiac output. The progressive inhibition of increases in heart rate, $LVdP/dt_{max}$ and cardiac output at higher levels of sympathetic activity can be explained by the $D_2$-mediated inhibition of catecholamine release from sympathetic nerve endings. In support of our observations, Girbes *et al*. (1992) showed that $D_1/D_2$ dopamine receptor agonist pro-drug ibopamine, which is converted into epinine, blunted the increases in circulating noradrenaline levels produced by bicycle exercise in man. Their finding that ibopamine did not blunt the exercise-induced tachycardia could have been due to direct $\beta_1/\beta_2$-adrenoceptor stimulation by the compound (Lopez-Sendon, 1990), a property which Z1046 appears to lack (Pocchiari *et al*., 1994; present study).

### *Conclusions*

The novel dopamine receptor agonist Z1046 is an effective blood pressure lowering agent that elicits minimal reflex activation of sympathetic nervous system in awake resting pigs. Systemic vasodilatation was not affected by combined $\alpha$- and $\beta$-adrenoceptor blockade, which is consistent with a predominantly $D_1$ receptor-dependent vasodilator mechanism. The hypotensive effect is maintained during treadmill exercise, during which systemic vasodilatation and a lower cardiac output both contribute to the blood pressure lowering actions of Z1046. The cardiovascular profile of this orally active compound warrants further investigation of this class of drugs in experimental and clinical hypertension.

This study has been made possible by a grant from Zambon Group S.P.A., Italy. The research of Dr D.J. Duncker has been made possible by a fellowship of the Royal Netherlands Academy of Arts and Sciences.

## References

DUNCKER, D.J., SAXENA, P.R. & VERDOUW, P.D. (1987). Systemic haemodynamic and beta-adrenoceptor antagonistic effects of bisoprolol in conscious pigs: a comparison with propranolol. *Arch. Int. Pharmacol. Ther*., **290,** 54–63.

DUNCKER, D.J., SAXENA, P.R. & VERDOUW, P.D. (1988). Systemic haemodynamics of dihydropyridine derivatives in conscious pigs with or without propranolol. *Eur. J. Pharmacol*., **156,** 401–409.

GIRBES, A.R., VELDHUISEN, D.J., VAN GREVINK, R.G., SMITH, A.J. & REITSMA, W.D. (1992). Effects of ibopamine on exercise-induced increase in norepinephrine in normal men. *J. Cardiovasc. Pharmacol*., **19,** 371–374.

GOLDBERG, L.I. (1972). Cardiovascular and renal actions of dopamine: potential clinical applications. *Pharmacol. Rev*., **24,** 1–29.

HIEBLE, J.P., OWEN, D.A.A., HARVEY, C.A., BLUMBERG, A.L., VALOCIK, R.E. & DEMARINIS, R.M. (1987). Hemodynamic effects of selective receptor agonists in the rat and dog. *Clin. Exp. Hypertension*, **9,** 889–912.

HUGHES, A.D. & SEVER, P.S. (1989). Action of fenoldopam. A selective dopamine ($D_1$) receptor agonist, on isolated human arteries. *Blood Vessels*, **26,** 119–127.

ISHIDA, T., LEWIS, R.M., HARLEY, C.J., ENTMAN, M.L. & FIELD, J.B. (1983). Comparison of hepatic extraction of insulin and glucagon in conscious and anesthetized dogs. *J. Endocrinol*., **74,** 800–802.

KOPIA G.A. & VALOCIK, R.E. (1989). Demonstration of specific dopamine-1 receptor-mediated coronary vasodilatation in the anaesthetized dogs. *J. Pharmacol. Exp. Ther*., **248,** 215–221.

LOPEZ-SENDON, J. (1990). Hemodynamic and neurohumoral effects of ibopamine in patients with chronic congestive heart failure. *Cardiology*, **77** (Suppl 5P), 9–21.

MARCHINI, F., PRADELLA, L., MIRAGOLI, G. & SEMERARO, C. (1994). Hemodynamic effects of Z1046, a new peripheral dopaminergic agent, in pentobarbitol anesthetized beagle dogs. *Can. J. Physiol. Pharmacol*., **72,** 131 (abstract).

POCCHIARI, F., ALLIEVI, L., ZANZOTTERA, D., MARCHINI, R. & SEMERARO, C. (1994). Z1046, a new potent and specific peripheral dopamine agonist. *Can. J. Physiol. Pharmacol*., **72,** 132 (abstract).

PRADELLA, L., BUSCHI, A., MARCHINI, F. & SEMERARO, C. (1995). The hemodynamic effects of Z1046 in anesthetized beagle dogs involves both D1-like and D2-like receptors stimulation. *J. Heart Failure*, **2,** 342 (abstract).

ROBERTS, J.G. (1980). Intravenous anaesthetic. In *The Circulation in Anaesthesia*, ed. Prys-Roberts, C. p. 460. Oxford: Blackwell Scientific Publications.

ROUSSEAU, M.F., KONSTAM, M., BENEDICT, C.R., DONKIER, J., GALANTI, L., MELIN, J., KINAN, D., AHN, S., KETELSLEGERS, J.M. & POULEUR, H. (1994). Progression of left ventricular dysfunction secondary to coronary artery disease, sustained neurohumoral activation and effects of ibopamine therapy during long-term therapy with angio-converting enzyme inhibitor. *Am. J. Cardiol*., **73,** 488–493.

ROUSSEAU, M.F., RAIGOSO, J., VAN EYLL, C., VAN MECHELEN, H., MUSSO, N.R., LOTTI, G. & POULEUR, H. (1992). Effects of intravenous epinine administration on left ventricular systolic performance, coronary hemadynamics, and circulating catecholamines in patients with heart failure. *J. Cardiovasc. Pharmacol.*, **19,** 155–162.

SCHUELKE, D.M., MARK, A.L., SCHMID, P.G. & ECKSTEIN, J.W. (1971). Coronary vasodilation produced by dopamine after adrenergic blockade. *J. Pharmacol. Exp. Ther.*, **176,** 320–327.

TODA, N. & HATANO, Y. (1979). Antagonism by droperidol or dopamine-induced relaxation in isolated dog arteries. *Eur. J. Pharmacol.*, **57,** 231–328.

UEDA, Y., YANO, S. & SAKANASHI, M. (1982). In vitro evidence for dopaminergic receptors in human renal artery. *J. Cardiovasc. Pharmacol.*, **4,** 76–81.

VAN WOERKENS, L.J., BAAS, N.R.A., VAN DER GIESSEN, W.J. & VERDOUW, P.D. (1992a). Cardiovascular effects of the novel potassium channel opener bimakalim in conscious pigs with and without myocardial infarction: A comparitive study with nicorandil. *Cardiovasc. Drugs Ther.*, **6,** 409–417.

VAN WOERKENS, L.J., BOOMSMA, F., MAN IN'T VELD, A.J., BEVERS, M.M. & VERDOUW, P.D. (1992c). Differential cardiovascular and neuroendocrine effects of epinine and dopamine in conscious pigs before and after adrenoceptor blockade. *Br. J. Pharmacol.*, **107,** 303–310.

VAN WOERKENS, L.J., DUNCKER, D.J., DEN BOER, M.O., MCFALLS, E.O., SASSEN, L.M.A., SAXENA, P.R. & VERDOUW, P.D. (1991). Evidence against a role for dopamine $D_1$ receptors in the porcine myocardium. *Br. J. Pharmacol.*, **104,** 246–250.

VAN WOERKENS, L.J., MAN IN'T VELD, A.J., VAN DER GIESSEN, W.J., VAN MEEGEN, J.R., BOOMSMA, F. & VERDOUW, P.D. (1992b). Effect of epinine on systemic hemodynamics and regional blood flows in conscious pigs. *J. Cardiovasc. Pharmacol.*, **19,** 580–586.

VATNER, S.F. (1978). Effects of anethesia on cardiovascular control mechanisms. *Environ. Health Perspect.*, **26,** 193–206.

VERDOUW, P.D., DUNCKER, D.J. & SAXENA, P.R. (1984). Poor vasoconstrictor response to adrenergic stimulation in the arteriovenous anastomoses present in the carotid vascular bed of young Yorkshire pigs. *Arch. Int. Pharmacodyn. Ther.*, **272,** 56–70.

VERDOUW, P.D., SASSEN, L.M.A., DUNCKER, D.J., SCHMEETS, I.O.L., RENSEN, R.J. & SAXENA, P.R. (1987). Nicorandil-induced changes in the distribution of cardiac output and coronary blood flow in pigs. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **336,** 352–358.

ZHAO, R., FENNELL, W.H. & ABEL, F.L. (1990). Effects of dopamine $D_1$ and dopamine $D_2$ receptor agonists on coronary and peripheral hemodynamics. *Eur. J. Pharmacol.*, **190,** 193–202.

*(Received October 16, 1996*
*Revised December 6, 1996*
*Accepted December 11, 1996)*