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Vasodilator actions of HA1077 *in vitro* and *in vivo* putatively mediated by the inhibition of protein kinase

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1 The *in vitro* and *in vivo* vasorelaxant effects of HA1077, 1-(5-isoquinolinesulphonyl)-homopiperazine HCl, a novel vasodilator were examined.

2 The inhibitory effects of HA1077 on contractile responses to various agonists were examined on strips of rabbit aorta. The concentration-response curves to 5-hydroxytryptamine, prostaglandin $F_{2\alpha}$, histamine, angiotensin II, noradrenaline and dopamine were concentration-dependently shifted to the right in the presence of HA1077 (0.3–3.0 μM).

3 The *in vivo* vasodilator effects of HA1077 were examined in the constant-pressure autoperfused coronary vascular bed of dogs. Intra-coronary administration of HA1077 (3–30 μg per dog) dose-dependently increased coronary blood flow (CBF), with no effect on mean blood pressure (MBP) or heart rate (HR). Intra-coronary infusion of atropine, propranolol or diphenhydramine did not modify the *in vivo* coronary vasodilator response to HA1077.

4 To determine the flow profile for HA1077 in dogs, blood flow in four vascular beds was measured, by use of noncannulating electromagnetic flow probes. HA1077 (0.01–0.3 mg kg^{-1}, i.v.) dose-dependently decreased MBP and increased vertebral blood flow (VBF), CBF, renal blood flow (RBF) and femoral blood flow (FBF).

5 A haemodynamic analysis showed that continuous i.v. infusion of HA1077 (0.01 and 0.033 mg kg^{-1} min^{-1}) dose-dependently decreased peripheral vascular resistance and increased cardiac output. There were no significant changes in right atrial pressure, *dP/dt* or ventricular minute work.

6 The effects of HA1077 on various enzymes considered to be related to the regulation of smooth muscle contraction were examined. HA1077 had little effect on cyclic nucleotide phosphodiesterases, yet it potently inhibited protein kinases such as cyclic nucleotide dependent protein kinases and Ca^{2+}/calmodulin dependent myosin light chain kinase.

7 The present study demonstrates that HA1077 is a novel type of arterial vasodilator.

Introduction

There is no definitive treatment for delayed cerebral vasospasm (Allen & Bahr, 1979; Gioia *et al.*, 1985) which often occurs following subarachnoid haemorrhage secondary to rupture of an intracranial aneurysm. We found that HA1077, 1-(5-isoquinolinesulphonyl)-homopiperazine HCl, and HA1004, N-(2-guanidinoethyl)-5-isoquinolinesulphonamide HCl, given intravenously, led to cerebral vasodilatation in a canine subarachnoid two-haemorrhage model (Takayasu *et al.*, 1986). Although their potential usefulness for the treatment of cerebral or coronary vasospasm in man has not been shown, the canine two-haemorrhage model used in our study mimics the vasospasm seen in patients with subarachnoid haemorrhage (Varsos *et al.*, 1983). Varsos *et al.* (1983) found no relief of cerebral vasospasm with comparable i.v. doses of nifedipine in the two-haemorrhage canine model.

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Nimodipine given intravenously produced a persistent hypotensive effect without affecting vasospasm in the two-haemorrhage dog model (Gioia *et al.*, 1985).

Because systemic i.v. application of HA1077 was more effective than HA1004 in a two-haemorrhage model (Takayasu *et al.*, 1986), the possible mechanism of action of HA1077 in the vascular-relaxation process was examined in our previous study (Asano *et al.*, 1987). HA1077 produced a competitive inhibition of Ca^{2+}-induced contractions of strips of depolarized aorta of the rabbit and significantly inhibited phenylephrine-induced contraction in Ca^{2+}-free solution. The contraction in response to adrenoceptor stimulation in Ca^{2+}-free solution is attributed almost entirely to release of intracellular Ca^{2+} (Fleckenstein, 1977; Weiss, 1981). Cultured vascular smooth muscle cells treated with HA1077 showed a dose-dependent loss or a decrease in size and length of the actin-containing microfilament structure (Sasaki *et al.*, 1987). Neither verapamil nor diltiazem in doses up to 100 μM induced reorganization of the actin microfilament structure. These results suggested that the mechanism of the vasodilator action of HA1077 differed from that of conventional calcium entry blockers and that its effect may be dependent upon an action on internal sequestering sites.

We describe here the pharmacology of HA1077 *in vitro* and its haemodynamic action, *in vivo*. Biochemical studies were also done to examine possible mechanisms of its vasodilator action.

Methods

Recording of mechanical responses

The aorta was excised from male rabbits (2–3 kg) and segments of the vessel were then trimmed of fat and connective tissue. The intimal surface of the preparation was rubbed gently to remove the endothelium and spirally cut strips 3 mm × 25 mm were prepared. The inhibitory effect of HA1077 on agonist-induced contractile response was studied in these preparations.

Each strip was mounted isometrically under 1.5 g of tension in a 20 ml tissue bath (37°C) containing normal physiological salt solution (pH 7.4) and aerated with 5% CO_2 in O_2. Force generation was monitored with an isometric transducer (Nihon Kohden, TB-611T) coupled to a polygraph recorder (Nihon Kohden, RM-6000). The normal physiological salt solution was composed of (mM): NaCl 115, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 10.0. A 1.5 h equilibration period was allowed before starting the experiments. During this period, the solutions were replaced every 30 min.

Cumulative concentration-response curves were constructed for 5-hydroxytryptamine, prostaglandin $F_{2\alpha}$, histamine, angiotensin II, noradrenaline and dopamine, as described by van Rossum (1963). Concentration-response curves for each agonist were constructed in the absence and in the presence of HA1077 (0.1–3 μM). Antagonist (HA1077) contact time was 5 min. Although the contact time was short, equilibrium was reached. The pD_2 values (i.e., the concentration of each agonist required to produce 50% of the maximal response) were calculated for each individual concentration-response curve to agonists. Details of the experimental procedures were as described by Hidaka *et al.* (1978).

In vivo *studies*

Mongrel dogs of either sex (10–15 kg) were anaesthetized with sodium pentobarbitone (35 mg kg^{-1}, i.v.). The trachea was intubated and ventilation was carried out with room air delivered from a respirator (Harvard, 607A). The ventilation rates (12–16 cycles min^{-1}) and tidal volumes (22–27 ml kg^{-1}) were adjusted to maintain the arterial blood pH, P_{CO_2} and P_{O_2}, within physiological limits. Body temperature was maintained at 37–38°C with a heating pad. Catheters were placed in the right femoral artery and vein to measure the pulsatile arterial blood pressure and i.v. injection of drug solution, respectively. Blood pressure (mmHg) was recorded from the femoral artery with a pressure transducer (Sanei, 45266). Mean blood pressure (MBP; mmHg) was calculated as diastolic blood pressure plus one third pulse pressure. Heart rate (HR; beats min^{-1}) was monitored with a cardiotachometer (Sanei, 2336A) triggered by a lead II electrocardiogram.

Arterial blood taken from the left femoral artery was led to the circumflex branch of the left coronary artery by a short extracorporeal loop. An electromagnetic flow probe of the extracorporeal type (Nihon Kohden, MFV-1200) was inserted into this circuit. Perfusion pressure was regulated by the use of pneumatic resistance. The coronary vascular bed was perfused extracorporeally under a controlled mean pressure at 100 mmHg. The drugs, in a volume of 0.03 ml, were given by a microinjector into a rubber tube connected close to the arterial cannula. Even though the drugs were given into the coronary artery and at low doses there were no alterations in systemic MBP or in HR. Heparin sodium (400 u kg^{-1}, i.v.) was administered to prevent clotting.

Mean blood flow in four vascular beds was measured with a noncannulating electromagnetic flow probe (Nihon Kohden, MFV-1100): left vertebral artery, circumflex branch of the left coronary artery, left renal artery and left femoral artery. A flow probe of adequate size was placed around each artery.

A Miller-microtransducer was introduced into the left ventricle via the left femoral artery to measure left ventricular systolic pressure, LVP (mmHg). Maximum left ventricular *dP/dt*, *dP/dt* (mmHg ms^{-1}) was determined by electronic differentiation of the pressure signal. Cardiac output, CO ($l\,min^{-1}$) was determined by the thermodilution technique using a Swan-Ganz catheter, a 5 ml iced injectate of 5% dextrose in H_2O, and a cardiac output computer (Edwards, 9520A). The Swan-Ganz catheter was introduced into a femoral vein and advanced to the pulmonary artery via the right ventricle and the right atrium. Right atrial pressure, RAP (mmHg) was measured with a pressure transducer (Sanei, 45266) via a catheter, which was introduced into a femoral vein and advanced to the right atrium. Additional values were obtained in the following manner: total peripheral resistance, TPR (mmHg min/l) = (MBP − RAP)/CO; left ventricular minute work, LVMW (Kp × m/min) = MBP × CO. TPR, CO and LVMW were expressed as 'index' (calculated for 10 kg body weight).

The vehicle for each drug was purified water or saline. The vehicle was administered to the controls and given in a volume equivalent to that used for the administered drugs. Significant changes in the cardiovascular parameters never occurred.

Enzyme assay and determinations

Myosin light chain, prepared by the method of Perrie & Perry (1970), was purified from chicken gizzard by the method of Adelstein & Klee (1981). The partially purified holoenzyme of adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-dependent protein kinase I (second DE-52 step) and its purified catalytic subunits were prepared from rabbit skeletal muscle, by the method of Beavo *et al.* (1974). Cyclic GMP-dependent protein kinase from pig lung was partially purified by the method of Kuo & Greengard (1974). Actomyosin from rabbit skeletal muscle was prepared according to Ebashi & Ebashi (1964).

Cyclic AMP-dependent protein kinase activity was assayed in a reaction mixture containing, in a final volume of 0.2 ml, 50 mM Tris-HCl (pH 7.0), 10 mM magnesium acetate, 2 mM EGTA, 1 μM cyclic AMP or absence of cyclic AMP, 3.3 to 20 μM [r-^{32}P]ATP (4×10^5 c.p.m.), 0.5 μg of the enzyme, 100 μg of histone H2B and each compound.

Cyclic GMP-dependent protein kinase activity was assayed in a reaction mixture containing, in a final volume of 0.2 ml, 50 mM Tris-HCl (pH 7.0), 50 mM magnesium acetate, 2 mM EGTA, 1 μM cyclic GMP or absence of cyclic GMP, 3.3 to 20 μM [r-^{32}P]ATP (4×10^5 c.p.m.), 2.4 μg of the enzyme, 100 μg of histone H2B and each compound.

Myosin light chain kinase activity was assayed, under the conditions described (Tanaka *et al.*, 1980), in a reaction mixture containing, in a final volume of 0.2 ml, 50 mM Tris-HCl (pH 7.0), 10 mM magnesium acetate, 0.1 mM calcium chloride or 1 mM EGTA, 100 ng of calmodulin, 5 to 100 μM [r-^{32}P]ATP (4×10^5 c.p.m.), 20 μM smooth muscle 20,000-dalton myosin light chain and 0.6 μg of myosin light chain kinase.

The mixture was incubated at 30°C for 5 min. The reaction was terminated by adding 1 ml of ice-cold 20% trichloroacetic acid after adding 500 μg of bovine serum albumin as a carrier protein. The sample was centrifuged at 3000 r.p.m. for 15 min, the pellet was resuspended in ice-cold 10% trichloroacetic acid solution and the centrifugation-resuspension cycle was repeated three times. The final pellet was dissolved in 1 ml of 1 N NaOH and radioactivity was measured with a liquid scintillation counter.

Actomyosin adenosine triphosphatase (ATPase) was assayed at 25°C in a volume of 0.2 ml containing 40 mM Tris-maleate buffer (pH 7.0), 60 mM KCl, 1 mM $MgCl_2$ 100 μM $CaCl_2$ and 0.2 to 1.0 mM ATP. The reaction was initiated by adding ATP and was terminated by adding 1 ml of 20% trichloroacetic acid. Inorganic phosphate was measured according to Martin & Dotty (1949).

Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Phosphodiesterase activity was measured as described (Hidaka & Shibuya, 1974). The reaction mixture (0.5 ml) contained 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, [^{3}H]-cyclic AMP or [^{3}H]-cyclic GMP, various concentrations of inhibitors and an appropriate amount of enzyme. After 15 min incubation at 30°C, the reaction was terminated by boiling for 5 min; 50 μg of snake venom was added and the mixture was incubated for another 10 min. Then 3 ml of water was added and the preparation applied to a small cation-exchange resin column (AG50-x 4, 200–400 mesh, 0.7 × 1.5 cm). The product, [^{3}H]-adenosine or [^{3}H]-guanosine, was eluted with 1.5 ml of 3 N ammonium hydroxide after the column had been washed with 15 ml of deionized water. The amount of product was determined in a liquid scintillation counter (Beckman, LS-7500).

Statistics

The values are given as mean ± s.e.mean. The significance of the difference was calculated by Student's t test or paired t test. P values of 0.05 or less were considered to represent significant differences.

Drugs and chemicals

HA1077 1-(5-isoquinolinesulphonyl)-homopiperazine HCl, HA1004, N-(2-guanidinoethyl)-5-isoquinolinesulphonamide HCl and trapidil HCl were synthesized and provided by Drs T. Sone and A. Morikawa in the Life Science Laboratories, Asahi Chemical Industry, Tokyo, Japan. The drugs used were nicardipine HCl (Yamanouchi, Tokyo, Japan), 5-hydroxytryptamine creatinine sulphate, prostaglandin $F_{2\alpha}$ tris salt, histamine diphosphate, angiotensin II acetate salt, noradrenaline bitartrate salt, dopamine HCl, acetylcholine chloride, diphenhydramine HCl, propranolol HCl, atropine sulphate salt, isoprenaline HCl (Sigma, St. Louis, MO, U.S.A.) and sodium pentobarbitone (Pittman-Moore, Indianapolis, IN, U.S.A.).

The doses of the drugs used for the *in vivo* experiments refer to the weights of the salts.

Results

Inhibition of agonist-induced contraction

5-Hydroxytryptamine, prostaglandin $F_{2\alpha}$, histamine, angiotensin II, noradrenaline and dopamine caused a concentration-dependent contraction in rabbit aortic strips. The contractile responses of the aorta to all agonists tested were significantly altered, concentration-dependently, in the presence of HA1077 (Figure 1 and Table 1). HA1077 in a concentration of 0.1 μM did not shift to the right the concentration-response curve of any agonist tested. With an increase in the concentration of HA1077 (0.3–3.0 μM), the concentration-response curves for agonists tested were shifted significantly, with a concomitant increase in pD_2 values or a decrease in the maximally developed tension.

HA1077 (1 μM) depressed significantly the maxima of concentration-response curves to 5-hydroxytryptamine (76.3 ± 6.6% vs. control, $P < 0.05$), with no effect on the pD_2 values (Table 1). HA1077 (1 μM) displaced to the right the concentration-response curves for all agonists tested, except for 5-hydroxytryptamine.

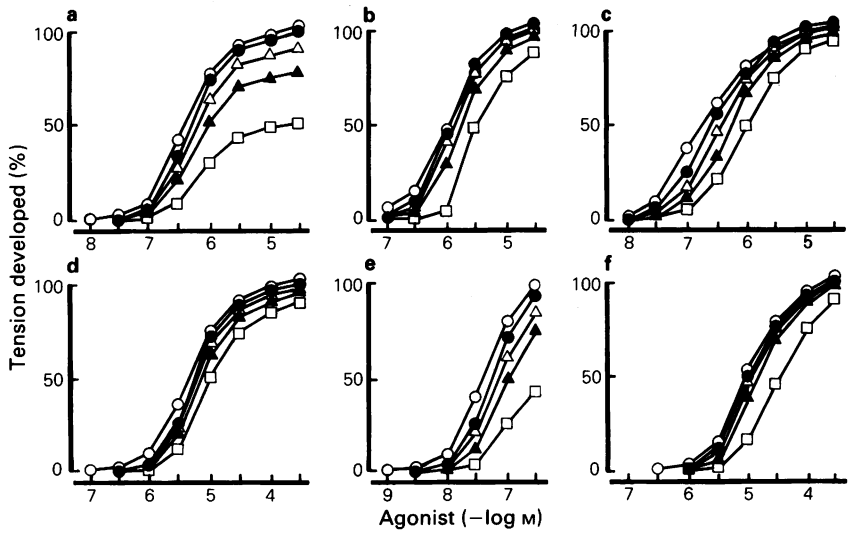

Figure 1 Effects of HA1077 on cumulative dose-response curves to 5-hydroxytryptamine (a, 5-HT), prostaglandin $F_{2\alpha}$ (b, $PGF_{2\alpha}$), noradrenaline (c, NA), histamine (d), angiotensin II (e, Ang II), and dopamine (f, DA) in rabbit aortic strips. Mean maximum responses were 2.99 ± 0.21 g ($n = 24$, 3×10^{-5} M 5-HT), 3.48 ± 0.13 g ($n = 24$, 3×10^{-5} M $PGF_{2\alpha}$), 3.70 ± 0.16 g ($n = 24$, 3×10^{-5} M NA), 5.25 ± 0.19 g ($n = 24$, 3×10^{-4} M histamine), 3.23 ± 0.20 g ($n = 24$, 3×10^{-7} M Ang II) and 4.37 ± 0.21 g ($n = 24$, 3×10^{-4} M DA), respectively. Each graph shows concentration-response curves obtained in the absence (○, $n = 24$) and in the presence of HA1077 [10^{-7} M (●, $n = 6$), 3×10^{-7} M (△, $n = 6$), 10^{-6} M (▲, $n = 6$) and 3×10^{-6} M (□, $n = 6$)]. For clarity only the mean values are given; s.e.mean ranges between 1 and 5%.

Table 1 Effects of HA1077 on the concentration-response curves of rabbit aortic strips for various agonists

Agonist	pD_2 *of HA1077* 0 μM	0.1 μM	1.0 μM	3.0 μM
			× 0.1 μM	
5-HT	4.40 ± 0.39	5.28 ± 0.89	17.32 ± 7.01	34.17 ± 7.55*
			× 1 μM	
$PGF_{2\alpha}$	1.13 ± 0.10	1.13 ± 0.16	1.85 ± 0.26*	3.70 ± 0.81*
			× 1 μM	
Histamine	4.77 ± 0.45	6.13 ± 1.15	7.37 ± 1.14*	11.85 ± 2.46*
			× 0.01 μM	
Ang II	4.13 ± 0.31	6.00 ± 0.86	13.52 ± 3.02*	24.07 ± 3.88*
			× 0.1 μM	
NA	1.97 ± 0.12	2.60 ± 0.29	5.72 ± 0.45*	11.53 ± 1.26*
			× 10 μM	
DA	0.91 ± 0.03 (n = 24)	1.08 ± 0.09 (n = 6)	1.49 ± 0.15* (n = 6)	3.58 ± 0.48* (n = 6)

Data are expressed as mean ± s.e.mean. ANOVA revealed significant differences between the effects of HA1077 on the concentration-response curves for each agonist ($P < 0.05$). The statistical significances between control and HA1077-treated groups are calculated by Student's t test (* $P < 0.05$). Abbreviations; 5-hydroxytryptamine, 5-HT; prostaglandin $F_{2\alpha}$, $PGF_{2\alpha}$; noradrenaline, NA; angiotensin II, Ang II; dopamine, DA.

Coronary circulation

Intra-coronary injection of HA1077, like other vasodilators, produced a dose-dependent increase in CBF, with no effect on MBP or HR (Figure 2). HA1077 (30 μg i.a.) produced an approximate 50% increase in CBF (predose CBF, 18.2 ± 2.8 ml min^{-1}; postdose CBF, 26.6 ± 3.3 ml min^{-1}). HA1077 was about 5 times more potent in increasing CBF than was HA1004. The rank order of potency for vasodilators tested was: nicardipine > HA1077 > trapidil > HA1004.

Continuous intra-coronary infusion of atropine (2 μg min^{-1}), propranolol (1 μg min^{-1}) and diphenhydramine (20 μg min^{-1}) led to no significant effect on the HA1077-induced vasodilatation. In contrast, infusion of either blocker completely or partially blocked the arterial response to acetylcholine (0.1 μg), isoprenaline (0.1 μg) and histamine (0.1 μg), respectively (Figure 3).

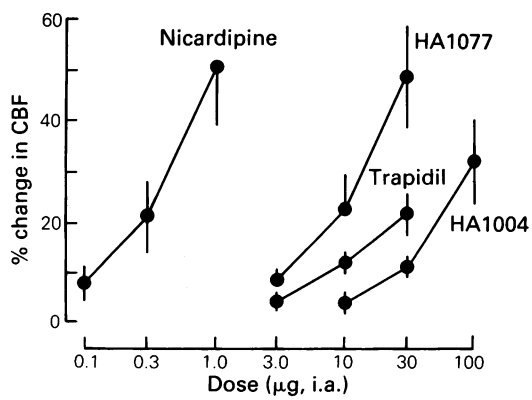

Figure 2 Effect of intra-coronary injection of HA1077 and various vasodilators on coronary blood flow (CBF) in pentobarbitone-anaesthetized dogs. The data used to calculate the percentage of changes were the maximum observed changes elicited after drug injection. Values represent mean of five experiments; vertical lines show s.e.mean.

Blood flow profile

HA1077 (0.01, 0.03, 0.1 and 0.3 mg kg^{-1}, bolus, i.v.) dose-dependently decreased MBP and increased HR, VBF, CBF, RBF and FBF (Table 2). In saline-treated animals, none of the parameters varied during the experiment.

Maximum changes in haemodynamic parameters occurred from 1 to 5 min after a bolus injection of HA1077 (Figure 4). The lowest dose of HA1077 (0.01 mg kg^{-1}, i.v.) produced significant increases in both VBF and CBF, with no significant effect on MBP, HR, RBF or FBF, although the increase in blood flow in the coronary and vertebral artery beds was minimal (<10%). There was a dose-related increase in both VBF and CBF, despite a significant decrease in MBP.

Nicardipine (3.0 μg kg^{-1}, i.v.) also produced a decrease in MBP and an increase in VBF, CBF and FBF. However, nicardipine produced no significant increases in RBF. In some dogs, there was a reduction in RBF after the i.v. administration of nicardipine (Table 2).

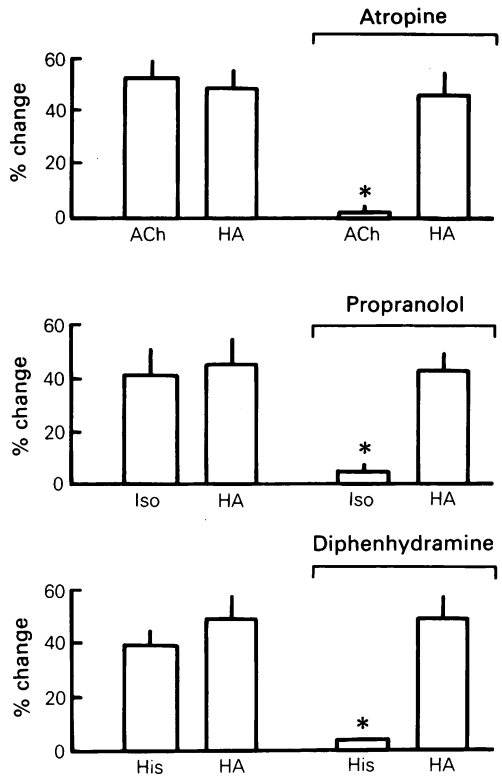

Figure 3 Maximal changes in coronary blood flow from control produced by relatively low intra-coronary injections of HA1077 (HA, 30 μg), acetylcholine (ACh, 0.1 μg), isoprenaline (Iso, 0.1 μg) and histamine (His, 0.1 μg) in pentobarbitone-anaesthetized dogs, in which specific blockers were infused. The continuous infusion of atropine (2 $\mu g\,min^{-1}$), propranolol (1 $\mu g\,min^{-1}$) and diphenhydramine (20 $\mu g\,min^{-1}$) failed to inhibit the HA1077-induced vasodilatation. Values represent mean ± s.e.mean of five experiments. The asterisks indicate significant differences from control (* $P < 0.05$; Student's t test).

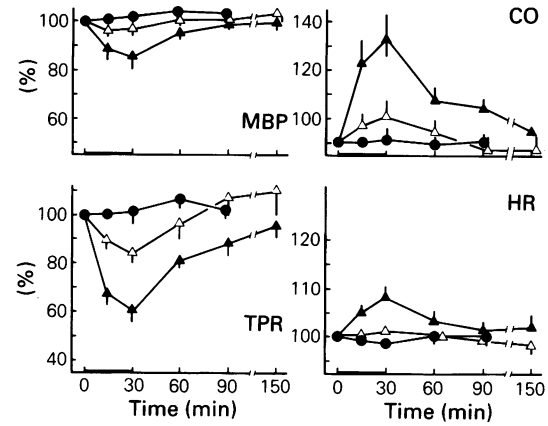

Figure 4 Time course of the effect of HA1077 infusion (0.01–0.033 $mg\,kg^{-1}\,min^{-1}$ for 30 min; total dose = 0.3–1.0 $mg\,kg^{-1}$) on mean blood pressure (MBP), cardiac output (CO), total peripheral resistance (TPR), and heart rate (HR) in pentobarbitone-anaesthetized dogs. HA1077 (●, saline; △, 0.3 $mg\,kg^{-1}$; ▲, 1.0 $mg\,kg^{-1}$) was infused intravenously over 30 min (indicated by bar), and each animal was observed for 150 min. Values represent mean of four to six experiments; s.e.mean shown by vertical lines.

Haemodynamics

HA1077 was administered by continuous i.v. infusion (0.01 and 0.033 $mg\,kg^{-1}\,min^{-1}$ for 30 min; total dose = 0.3 and 1.0 $mg\,kg^{-1}$). Figure 4 shows the time courses of the changes in MBP, cardiac output, total peripheral resistance and HR in the saline infusion and the low (0.01 $mg\,kg^{-1}\,min^{-1}$), and high (0.033 $mg\,kg^{-1}\,min^{-1}$) dose groups. In the saline-treated animals (0.1 $ml\,kg^{-1}\,min^{-1}$ for 30 min; total dose = 3 $ml\,kg^{-1}$), the parameters did not vary during the experiment. In dogs given a low dose of HA1077 (total dose = 0.3 $mg\,kg^{-1}$), there was no immediate hypotensive episode because HA1077 was infused over a 30 min period rather than as a bolus. The bolus administration of HA1077 (0.3 $mg\,kg^{-1}$) caused a severe hypotension (Figure 5).

The maximal increase in cardiac output occurred at 30 min after start of the i.v. infusion and it increased from 1.74 $l\,min^{-1}$ at basal to 2.38 $l\,min^{-1}$ within 30 min of the start of the high-dose HA1077 infusion. The calculated total peripheral resistance reflects these changes; it fell significantly in a dose-dependent manner following the infusion of HA1077.

The haemodynamic response to i.v. infusion of HA1077 in dogs is summarized in Table 3. HA1077 produced a significant fall in MBP, left ventricular systolic pressure and total peripheral resistance with an increase in HR and cardiac output. There were no significant changes in right atrial pressure, dP/dt or left ventricular minute work.

Protein kinase inhibition

The effect of HA1077 on various enzymes considered to be related to the regulation of smooth muscle contraction were examined (Table 4). HA1077 had little effect on cyclic nucleotide phosphodiesterases, but the protein kinases were potently inhibited. The K_i values of HA1077 for cyclic nucleotide-dependent protein kinases and Ca^{2+}/calmodulin-dependent myosin light chain kinase were 1.6 and 36 μM, respectively. Actomyosin ATPase activity was not inhibited in concentrations up to 100 μM.

Table 2 Change in blood pressure, heart rate and blood flow after bolus i.v. administration of HA1077 to pentobarbitone-anaesthetized dogs

Parameter		*HA1077* 0.01 mg kg^{-1} (n = 7)	*HA1077* 0.03 mg kg^{-1} (n = 7)	*HA1077* 0.1 mg kg^{-1} (n = 12)	*HA1077* 0.3 mg kg^{-1} (n = 12)	*Nicardipine* 3.0 μg kg^{-1} (n = 12)
MBP (mmHg)	Base	127.9 ± 6.9	127.3 ± 7.7	121.0 ± 5.6	13.4 ± 5.1	105.9 ± 4.0
	Δmax	0.0 ± 0.0	−4.0 ± 0.6*	−14.1 ± 1.5*	−31.5 ± 3.0*	−22.3 ± 2.8*
HR (beats min^{-1})	Base	124.0 ± 4.0	122.0 ± 4.0	122.6 ± 3.8	114.8 ± 3.7	112.5 ± 3.1
	Δmax	0.1 ± 0.1	1.3 ± 0.5*	5.9 ± 1.0*	9.3 ± 1.2*	4.5 ± 1.7*
VBF (ml min^{-1})	Base	18.2 ± 2.8	18.2 ± 3.2	23.1 ± 5.1	21.7 ± 6.0	18.1 ± 4.3
	Δmax	1.7 ± 0.6*	4.0 ± 1.1*	17.6 ± 4.3*	22.2 ± 5.4*	20.3 ± 3.0*
CBF (ml min^{-1})	Base	32.2 ± 9.6	27.8 ± 8.4	38.7 ± 8.6	35.1 ± 7.8	23.9 ± 4.5
	Δmax	0.8 ± 0.2*	4.5 ± 1.7*	11.8 ± 2.2*	22.6 ± 3.1*	23.4 ± 4.7*
RBF (ml min^{-1})	Base	78.9 ± 12.9	97.0 ± 8.3	104.1 ± 9.7	104.1 ± 7.1	109.3 ± 12.6
	Δmax	1.1 ± 0.6	10.0 ± 1.4*	19.8 ± 3.0*	23.8 ± 4.2*	−2.0 ± 8.9
FBF (ml min^{-1})	Base	53.5 ± 14.5	51.0 ± 22.0	62.3 ± 10.7	47.3 ± 9.7	31.0 ± 7.0
	Δmax	1.0 ± 1.0	4.5 ± 0.5*	11.3 ± 2.1*	14.7 ± 6.2*	15.5 ± 3.1*

Data are expressed as mean ± s.e.mean. Number in parentheses represents the number of dogs. The asterisks indicate significant differences from representative control before HA1077 administration (* $P < 0.05$; paired t test). Abbreviations: MBP, mean blood pressure; HR, heat rate; VBF, vertebral blood flow; CBF, coronary blood flow; RBF, renal blood flow; FBF, femoral blood flow.

Discussion

The present *in vitro* study revealed that HA1077 is a vasodilator with a potentially novel mechanism of action. The concentrations of HA1077 inhibiting agonist-induced contractions of rabbit aorta were similar to those inhibiting protein kinase.

HA1077 inhibited various agonist-induced vascular contractions, at concentrations of 0.3–3.0 μM (Figure 1). These results are consistent with a foregoing assumption that the mechanism of the vasodilator action of HA1077 differed from that of conventional calcium entry blockers. These blockers selectively shifted concentration-response curves to KCl, as opposed to concentration-response curves to receptor agonists such as noradrenaline and prostaglandin $F_{2\alpha}$ (Asano & Hidaka, 1984).

Inhibition of the 5-hydroxytryptamine-induced

Table 3 Summary of effects of i.v. infusion of HA1077 on haemodynamic parameters in pentobarbitone-anaesthetized dogs

Parameter		*HA1077* (mg kg^{-1} min^{-1}) 0	0.01	0.033
MBP (mmHg)	Base	131.7 ± 6.0	116.8 ± 8.5	122.2 ± 9.2
	Δmax	1.3 ± 1.2	−8.3 ± 1.5*	−15.8 ± 4.8*
HR (beats min^{-1})	Base	142.2 ± 4.8	126.8 ± 8.7	126.4 ± 11.0
	Δmax	−3.0 ± 0.6	1.4 ± 1.8	11.2 ± 3.4*
RAP (mmHg)	Base	5.4 ± 0.5	10.6 ± 4.9	5.4 ± 0.5
	Δmax	0.0 ± 0.0	0.4 ± 0.4	0.2 ± 0.2
LVP (mmHg)	Base	138.4 ± 6.5	127.8 ± 10.3	131.8 ± 11.2
	Δmax	2.4 ± 1.4	−9.8 ± 1.8*	−16.0 ± 3.8*
dP/dt (mmHg ms^{-1})	Base	2.4 ± 0.2	2.4 ± 0.3	2.5 ± 0.2
	Δmax	0.0 ± 0.0	0.0 ± 0.1	0.4 ± 0.3
CO (l min^{-1})	Base	1.18 ± 0.12	1.55 ± 0.40	1.74 ± 0.47
	Δmax	0.00 ± 0.04	0.22 ± 0.10	0.64 ± 0.09*
TPR (kdyn s cm^{-1})	Base	9.1 ± 1.3	6.8 ± 1.3	6.7 ± 1.2
	Δmax	0.1 ± 0.4	−1.1 ± 0.3*	−2.8 ± 0.7*
LVMW (kp m min^{-1})	Base	2.08 ± 0.14	2.32 ± 0.40	2.71 ± 0.50
	Δmax	0.03 ± 0.08	0.16 ± 0.12	0.49 ± 0.40

Data are expressed as mean ± s.e.mean of five experiments. CO, TPR and LVMW are expressed as 'index' (10 kg^{-1}). The asterisks indicate significant differences from representative control before saline or HA1077 administration (* $P < 0.05$; paired t test). For further abbreviations see 'Methods'.

Table 4 Effect of HA1077 on various enzymes related to regulation of smooth muscle contraction

Regulators of intracellular Ca^{2+}	K_i μM
Cyclic AMP-dependent protein kinase	1.6
Cyclic GMP-dependent protein kinase	1.6
Ca^{2+}/calmodulin-dependent myosin light chain kinase	36
Actomyosin ATPase	>100
Cyclic AMP phosphodiesterase	>100
Cyclic GMP phosphodiesterase	>100

Values of K_i were obtained from Dixon plots using three concentrations of substrate and four to five concentrations of HA1077.

contraction differs, from findings with other agonists. We have no obvious explanation for the different profile against 5-hydroxytryptamine.

The present *in vivo* study confirms that HA1077 has a potent dilator effect on both cerebral and coronary vascular beds in dogs. Intra-coronary administration of HA1077 (3–30 μg) dose-dependently increased CBF, with no effect on MBP or HR. We have already reported that the intra-vertebral administration of HA1077, at the same doses, increased VBF (Asano *et al.*, 1987).

The vasodilator effects of the intra-coronary injections of HA1077 to anaesthetized dogs were not altered by atropine, propranolol, or diphenhydramine treatment. The *in vivo* action of HA1077 is clearly different from other agents such as acetylcholine, isoprenaline, or histamine. Similarly, the intra-vertebral injection of HA1077 dose-dependently increased VBF, which was not prevented by these blockers (data not shown).

Intra-coronary administration of HA1077 was significantly more potent in increasing CBF than was HA1004 (Figure 2). This is consistent with previous observations that systemic i.v. application of HA1077 was more effective than HA1004 in cases of canine chronic vasospasm (Takayasu *et al.*, 1986).

One notable difference between the blood flow profile for HA1077 and nicardipine concerned renal haemodynamic alterations. In our previous study, nicardipine (0.01–10 μg kg^{-1}, i.v.) caused a dose-dependent decrease in RBF, with a significant reduction in MBP (Asano & Hidaka, 1985). Hof (1983) also found that the increase in RBF produced by nicardipine was not significant. In addition, there was a reduction in RBF after i.v. injections of nicardipine (3 μg kg^{-1}), in some dogs in the present study. On the other hand, i.v. administration of HA1077 caused a significant and dose-dependent increase in RBF, despite a reduction in MBP (Table 2).

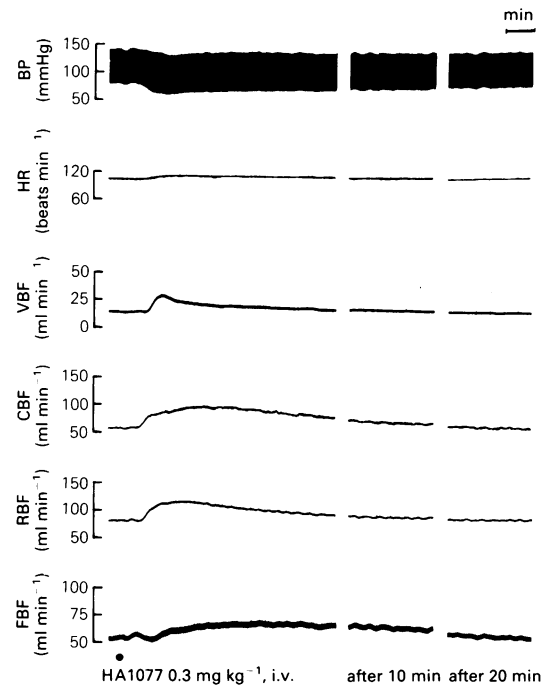

Figure 5 Effects of bolus i.v. administration of HA1077 on blood pressure (BP), heart rate (HR), vertebral blood flow (VBF), coronary blood flow (CBF), renal blood flow (RBF) and femoral blood flow (FBF) as illustrated by polygraph tracings obtained from a typical experiment. HA1077 (0.3 mg kg^{-1}) was given i.v. as bolus administration.

Infusion of HA1077 i.v. decreased total peripheral resistance and increased cardiac output, despite significant decreases in MBP. HA1077 given as an i.v. bolus had qualitatively the same effects on these cardiovascular haemodynamic parameters as did the i.v. infusion (data not shown). Haemodynamic analysis indicates a direct relaxant effect of HA1077 on arterial vascular smooth muscle.

Basal values for right atrial pressure in Table 3 seem rather high. One possible explanation is that cardiac output was determined simultaneously by the thermodilution method. Here, a Swan-Ganz catheter was introduced into the pulmonary artery via the right ventricle and the right atrium as described in Methods.

Analysis of the present biochemical effects of HA1077 revealed that this drug inhibits cyclic nucleotide-dependent protein kinases at concentrations similar to those causing inhibition of contractile response to vasoconstrictor agonists. These results suggest that the vasodilator effect of HA1077 may be related to its ability to inhibit specifically

cyclic nucleotide-dependent protein kinases. This would appear to be one of the major findings of the present study. However, there are data suggesting that activation of these kinases is related to vascular smooth muscle relaxation (Kuo & Greengard, 1969; Adelstein & Eisenberg, 1980). The relaxation of vascular smooth muscle induced by many vasodilators is thought to be mediated by cyclic AMP and/or cyclic GMP accumulation, and subsequent activation of cyclic nucleotide-dependent protein kinases (Adelstein & Eisenberg, 1980). Thus, although the present data suggest a putative involvement of cyclic nucleotide-dependent protein kinases in the HA1077-induced vasodilatation in vascular smooth muscle, more evidence is required to establish clearly any functional relationship.

The concentrations of HA1077 inhibiting cyclic AMP- and cyclic GMP-dependent protein kinases are similar to those inhibiting the agonist-induced contraction of vascular smooth muscle. Protein phosphorylation plays a significant role in modulating various cellular processes. The importance of protein kinase activity in the vascular contraction-relaxation cycle is now being investigated with HA1077.

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