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Antianginal effects of hydroxyfasudil, a Rho-kinase inhibitor, in a canine model of effort angina

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1 The effects of Rho-kinase inhibitor, fasudil, and of a more specific Rho-kinase inhibitor, hydroxyfasudil, on pacing-induced myocardial ischaemia were determined in anaesthetized open-chest dogs.

2 The dogs were subjected to left anterior descending coronary artery (LAD) stenosis producing a sufficient ischaemia as measured by ST-segment depression on electrocardiograms only when the hearts were paced 60 beats min^{-1} above the baseline. After a recovery (nonpacing) period, drugs or saline were infused intravenously over 30 min. The animals were again subjected to 5 min of pacing 25 min after the initiation of the treatment. Hydroxyfasudil (0.1 and 0.3 mg kg^{-1}) and fasudil (0.3 mg kg^{-1}) suppressed the ST-segment depression.

3 Hydroxyfasudil and fasudil also increased the regional blood flow of the LAD perfused endomyocardium region in the canine model of effort angina.

4 To determine the flow profile for hydroxyfasudil in dogs, blood flow in three vascular beds was measured. Hydroxyfasudil (0.3 mg kg^{-1} for 30 min) significantly increased coronary blood flow and vertebral blood flow, without significantly changing the femoral blood flow.

5 Hydroxyfasudil had no inotropic or chronotropic effect on the isolated hearts of guinea-pigs. Hydroxyfasudil (2 mg kg^{-1} for 20 min) did not affect the PR or QTc interval in anaesthetized dogs.

6 Inhibition of Rho-kinase appears to protect myocardium subjected to pacing-induced ischaemia through the increase in the regional myocardial blood flow. Hydroxyfasudil may be categorized as a novel type of anti-anginal drug, without any inotropic or chronotropic effects.

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Keywords: effort angina; Rho-kinase inhibitor; hydroxyfasudil; fasudil

Abbreviations: CBF, coronary blood flow; ECG, electrocardiograms; FBF, femoral blood flow; HR, heart rate; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; MBP, mean arterial blood pressure; MLC, myosin light chain; MLCK, myosin light chain kinase; PKC, protein kinase C; RMBF, regional myocardial blood flow; VBF, vertebral blood flow

Introduction

Rho-kinase has been shown to be involved in myosin light chain (MLC) phosphorylation in vascular smooth muscle cells or in stress fibre formation in non-smooth muscle cells (Uehata *et al*., 1997; Hori & Karaki, 1998). Rho-kinase is thought to play an important role in the regulation of smooth muscle contraction and cell migration (Hori & Karaki, 1998; Maekawa *et al*., 1999; Niggli, 1999).

Hydroxyfasudil, the main active metabolite of fasudil (Satoh *et al*., 2001), is a Rho-kinase inhibitor (Shimokawa *et al*., 1999). Hydroxyfasudil has a potent vasodilator effect on coronary vascular bed and inhibits the enhanced MLC mono- and diphosphorylation (Shimokawa *et al*., 1999). Hydroxyfasudil also inhibits various chemoattractant-induced migration of neutrophils (Satoh *et al*., 1999). Fasudil, the parent drug of hydroxyfasudil, is also a Rho-kinase inhibitor (Uehata *et al*., 1997; Nagumo *et al*., 2000; Davies *et al*., 2000), and has a potent vasodilator effect on coronary vascular bed and inhibits cell migration (Katsumata *et al*., 1997; Satoh *et al*., 1999; Negoro *et al*., 1999; Miyata *et al*., 2000). Fasudil significantly dilated spastic arteries in a swine model of coronary artery spasm and prevented myocardial injury after ischaemia (Katsumata *et al*., 1997; Yamamoto *et al*., 2000). It was reported that hydroxyfasudil was more selective than fasudil as an inhibitor of Rho-kinase (Shimokawa *et al*., 1999). Therefore, hydroxyfasudil is expected to prevent the pathogenesis of myocardial injury after ischaemia.

Angina pectoris is a clinical syndrome that occurs when there is an imbalance between the supply and demand of myocardial oxygen. Since the change in the ST-segments on electrocardiograms (ECG) is closely related to the change in the oxygen supply/demand rate, this can be a useful tool for the diagnosis of angina pectoris. Coronary vasodilators are thought to be effective in relieving vasoconstriction, improving coronary blood flow and increasing oxygen supply (Parmley, 1998). In the present study, we investigated the effects of hydroxyfasudil on the ST-segment changes induced by the left anterior descending coronary artery (LAD) stenosis plus pacing and determined whether such a

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protective effect occurs with concomitant changes in myocardial blood flow. The pharmacological profile of hydroxyfasudil in the cardiovascular system was also evaluated.

Methods

Myocardial ischaemia caused by partial occlusion of the coronary artery and pacing in dogs

Mongrel dogs of either sex (9–24 kg) were anaesthetized with pentobarbitone sodium (35 mg kg^{-1} i.v.). Ventilation was through an endotracheal tube using room air delivered by a respirator (Harvard, Model NSH-34RH). Tidal volume (22 ml kg^{-1}) and the rate of ventilation (16–30 cycles min^{-1}) were adjusted to maintain the arterial blood pH, pCO_2 and pO_2, within physiological ranges. A polyethylene catheter was inserted into the right femoral artery to monitor the mean arterial blood pressure (MBP) and heart rate (HR), and a second catheter was inserted into the left femoral artery to obtain arterial blood samples. A thoracotomy was performed, and LAD was isolated at the origin and a silk ligature was placed around it for partial occlusion of LAD. An electromagnetic flow probe (Nihon Kohden, Model MFV-2100) was placed distal to the ligature. Mean blood flow in the left circumflex coronary artery (LCX) was also measured using an electromagnetic flow probe. For pacing of the heart, a pair of electrodes were positioned in the right auricular appendage. The pacing was accomplished with a driving stimulation using pulses of 2–3 V and 2 msec duration (Nihon Kohden, Electric stimulator, SEN-7103). After completing the preparation and a stabilization period, the LAD was partially occluded to reduce the coronary blood flow by two-thirds of the basal flow. The heart was then driven for 5 min at 60 beats min^{-1} above the baseline heart rate. After a recovery (nonpacing) period, drugs or saline was infused intravenously over 30 min. The animals were subjected to 5 min periods of pacing again 25 min after the initiation of the treatment. A lead II electrocardiogram was recorded continuously during the experiments.

Measurement of regional myocardial blood flow (RMBF) in the canine model of effort angina

A catheter was inserted into the left atrium through the left atrial appendage for administration of microspheres. Blood flow was measured with three types of 6×10^6 coloured microspheres (o.d. 15.5 μm, dye-trak, Triton Technology Inc.) before, and at 25 and 30 min after starting the drug administration. A reference blood sample was obtained at a rate of 6 ml min^{-1} starting 10 s before the injection of microspheres and continuing until 90 s after the initiation of injection (total collection time 100 s). Twenty minutes after completion of microsphere injection, the heart was removed. A

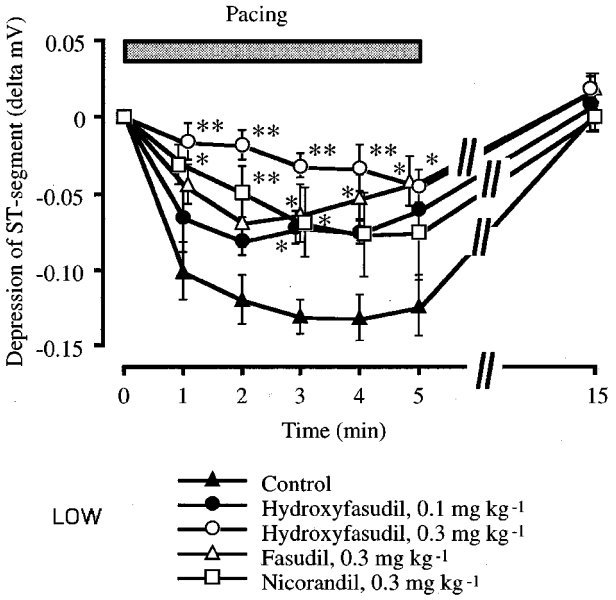

Figure 1 Effect of saline, hydroxyfasudil (0.1 mg kg^{-1}, 0.3 mg kg^{-1}), fasudil (0.3 mg kg^{-1}) or nicorandil (0.3 mg kg^{-1}) on tachy-pacing-induced ST-segment depression in dogs. Pacing was started at time 0. Each data point represents the mean ± s.e.mean of six experiments. The asterisks indicate a significant difference from the control (**$P<0.01$, *$P<0.05$: Dunnett's test).

Table 1 Effects of hydroxyfasudil and fasudil on MBP and HR before, during or after pacing-induced ischaemia in dogs

Treatment	*Dose (mg kg^{-1})*	*Prepace[a] (%)*	*Pace[b] (%)*	*Postpace[c] (%)*
MBP				
Control		100.7 ± 1.9	94.0 ± 2.5	103.2 ± 3.5
Hydroxyfasudil	0.1	97.8 ± 2.7	101.2 ± 5.2	100.1 + 4.4
	0.3	90.6 ± 2.1**	82.2 ± 4.6	91.7 ± 1.7*
Fasudil	0.3	93.8 ± 1.7	88.0 ± 3.4	94.4 ± 1.9
Nicorandil	0.3	92.4 ± 1.8*	87.9 ± 3.3	94.4 ± 2.5
HR				
Control		99.2 ± 4.3	142.6 ± 8.2	95.9 ± 4.5
Hydroxyfasudil	0.1	97.7 ± 1.0	141.0 ± 4.4	96.9 ± 1.8
	0.3	101.1 ± 1.7	147.3 ± 5.1	99.4 ± 1.3
Fasudil	0.3	98.8 ± 0.9	140.4 ± 1.5	96.3 ± 1.0
Nicorandil	0.3	96.9 ± 0.5	137.7 ± 1.2	93.5 ± 1.3

MBP or HR is expressed as a percentage of the change from pre-dose baseline values (−25 min). Drugs were infused intravenously over 30 min and animals were subjected to 5 min period of pacing 25 min after the initiation of drugs treatment. Values are expressed as mean ± s.e.mean of six experiments. MBP, mean blood preasure; HR, heart rate. *$P<0.05$, **$P<0.01$ vs control. [a]: the value before pacing (0 min). [b]: the value at 5 min after starting the pacing. [c]: the value at 10 min after finishing the pacing.

catheter was inserted into LAD and 1% Evans blue (0.3–0.7 ml) was infused. Tissue samples from the stained portion (the LAD perfused region) and the unstained portion of the left ventricle were obtained from each dog. After tissue digestion with a 4 mol l^{-1} KOH solution containing 2% Tween 80, microspheres were recovered by vacuum filtration (10 μm pore size polyester filter membranes). The reference blood samples were processed in a similar manner. Dye was recovered from the microspheres by adding 250 μl N,N-dimethylformamide as a solvent. For microsphere quantification, the dye content was then determined at a wavelength of 450, 530 or 670 nm with a spectrophotometer. Separation of overlapping spectra was performed with a matrix inversion technique.

Blood flow profile

Mongrel dogs of either sex (10–24 kg) were anaesthetized with pentobarbitone sodium (35 mg kg^{-1} i.v.). Ventilation was through an endotracheal tube using room air. A polyethylene catheter was inserted into the right femoral artery to monitor MBP and HR. Mean blood flow in the right vertebral artery, LCX and left femoral artery was measured with a noncannulating electromagnetic flow probe (Nihon Kohden, MFV-2100). A flow probe of adequate size was placed around each artery.

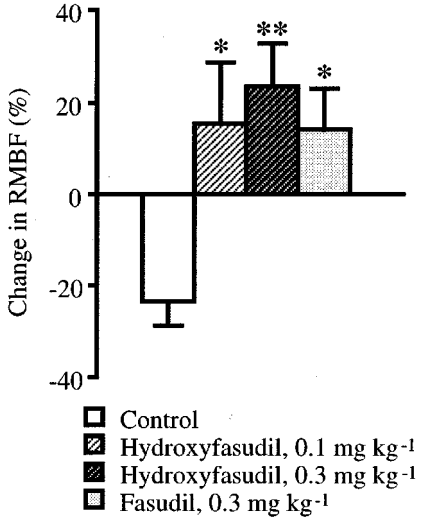

Figure 2 Effect of saline, hydroxyfasudil (0.1 mg kg^{-1}, 0.3 mg kg^{-1}) or fasudil (0.3 mg kg^{-1}) on regional myocardial blood flow (RMBF) of the left anterior descending coronary artery perfused endomyocardium region in dogs. Each column represents the mean ± s.e.mean of six experiments. The asterisks indicate a significant difference from the control (**$P<0.01$, *$P<0.05$: Dunnett's test).

Cardiac conduction system

Mongrel dogs of either sex (8–23 kg) were anaesthetized with pentobarbitone sodium (30 mg kg^{-1}, i.v.). Dogs were intubated and were allowed to breathe spontaneously in room air. A polyethylene catheter was inserted into the right femoral artery to monitor MBP. HR was monitored with a cardiotachometer triggered by a lead II electrocardiogram. Electrodes were placed on the four limbs for monitoring the surface ECG. The rate-corrected interval (QTc) was calculated using the following formula: $QTc = QT/RR^{1/2}$.

Isolated guinea-pig atria

Male guinea-pigs (350–400 g) were used. They were sacrificed and the heart was quickly removed. The right and left atria were excised, and the preparations were mounted in organ baths that contained modified Krebs-Ringer solution maintained at 37°C and oxygenated with 95% O_2 and 5% CO_2. The resting tension of the right and left atrium was adjusted to 0.5 g. The right atrium was allowed to beat spontaneously and the heart rate was measured using a tachometer. The left atrium was stimulated electrically at a frequency of 2 Hz by square-wave pulses of 5 msec duration and a voltage intensity that was 1.2 fold the threshold. Concentration-response curves were constructed by adding hydroxyfasudil at concentrations of 10^{-7}–10^{-4} M to the organ baths in a cumulative manner.

Drugs

The drugs used were fasudil, hydroxyfasudil (Asahi Kasei Corporation, Tokyo, Japan) and pentobarbitone sodium (Pitmann-Moore, IL, U.S.A.).

Table 2 Effects of hydroxyfasudil and fasudil on regional myocardial blood flow (RMBF) in unstained and stained regions of the left ventricle

	Dose	*Unstained region*		*Stained region*	
Treatment	*(mg kg^{-1})*	*ENDO (%)*	*EPI (%)*	*ENDO(%)*	*EPI(%)*
Pre-pace					
Control		93.2±2.8	96.6±6.3	101.8±7.7	98.8±2.8
Hydroxyfasudil	0.1	121.2±11.5	131.1±9.1	103.7±2.7	107.8±3.5
	0.3	108.9±4.1	139.4±7.7*	101.6±8.1	98.2±6.1
Fasudil	0.3	113.1±10.9	123.0±15.0	110.3±10.6	104.0±6.5
Pace					
Control		103.3±7.3	114.5±5.2	76.4±5.1	105.5±9.2
Hydroxyfasudil	0.1	125.0±13.1	171.9±11.9**	115.4±13.2*	159.9±18.4*
	0.3	132.4±13.2	172.5±18.1**	123.6±9.1**	141.7±17.6
Fasudil	0.3	122.1±6.6	143.9±10.1	114.0±9.0*	122.9±6.1

RMBF is expressed as a percentage of the change from the pre-dose baseline values. Drugs were infused intravenously over 30 min and animals were subjected to 5 min period of pacing 25 min after the initiation of drugs treatment. Values are expressed as mean ± s.e.mean of six experiments. ENDO, endocardium; EPI, epicardium. *$P<0.05$, **$P<0.01$ vs control.

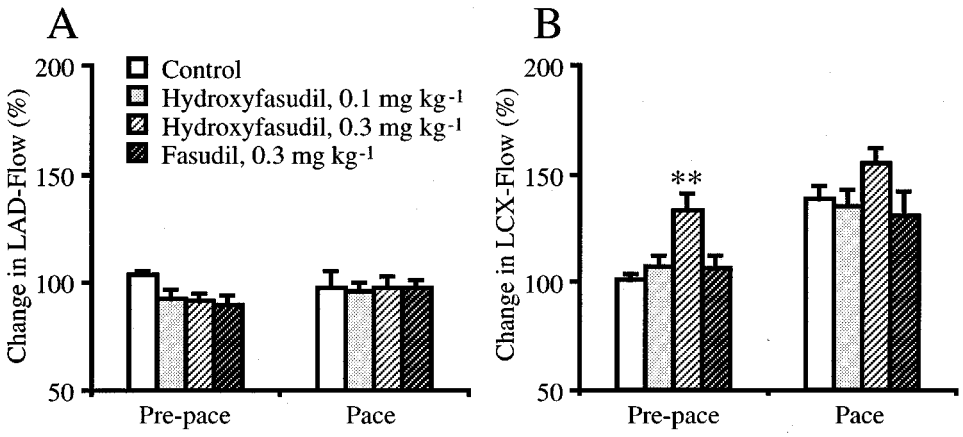

Figure 3 Effect of saline, hydroxyfasudil (0.1 mg kg^{-1}, 0.3 mg kg^{-1}) or fasudil (0.3 mg kg^{-1}) on the blood flow through the left anterior descending coronary artery (LAD) (A) and through the left circumflex coronary artery (LCX) (B). Each column represents the mean ± s.e.mean of six experiments. The asterisks indicate a significant difference from the control (**$P<0.01$: Dunnett's test).

Statistics

Values are expressed as mean ± s.e.mean. The significance of difference was calculated by Student's *t* test or Dunnett's test. *P* values of less than 0.05 were considered to be statistically significant.

Results

Effects on ST-segment depression

In anaesthetized dogs, pacing plus LAD stenosis resulted in ST-segment depression. After cessation of pacing, ST-segment depression returned to prepacing baseline levels. Hydroxyfasudil (0.1 and 0.3 mg kg^{-1}) dose-dependently attenuated the ST-segment depression induced by pacing (Figure 1). For example, at 3 min after the onset of pacing, the ST-segment depression was -0.131 ± 0.011 mV in the control group; the difference was significant compared with the ST-segment depression of -0.072 ± 0.011 and -0.032 ± 0.008 mV observed in dogs treated with 0.1 and 0.3 mg kg^{-1} of hydroxyfasudil, respectively. Fasudil (0.3 mg kg^{-1}) and nicorandil (0.3 mg kg^{-1}) also significantly attenuated the ST-segment depression (Figure 1). MBP and HR are expressed as a percentage of the change from the baseline value (Table 1). Pacing resulted in HR elevation to equivalent levels in all groups. MBP remained constant throughout the pacing period for all groups. Twenty-five minutes after the initiation of hydroxyfasudil (0.3 mg kg^{-1}) or nicorandil (0.3 mg kg^{-1}) infusion, MBP was slightly but significantly decreased (Table 1).

Effects on RMBF

RMBF is expressed as a percentage of the change from the baseline value. After pacing was superimposed on the LAD stenosis, RMBF slightly increased when compared with the prepacing value in the unstained regions and in the LAD perfused epicardium region (Table 2). In contrast, RMBF decreased by 24% in the LAD perfused endomyocardium region (Table 2). Hydroxyfasudil (0.1 and 0.3 mg kg^{-1}) and fasudil (0.3 mg kg^{-1}) significantly improved the RMBF in the LAD perfused endomyocardium region (Figure 2 and Table 2).

The blood flow through LAD remained constant by the infusion of hydroxyfasudil or fasudil, and pacing (Figure 3). Twenty-five minutes after the initiation of the drug treatment, hydroxyfasudil (0.1 mg kg^{-1}) or fasudil (0.3 mg kg^{-1}) did not affect the blood flow through LCX, but hydroxyfasudil (0.3 mg kg^{-1}) significantly increased the LCX flow. Pacing resulted in the increase in the LCX blood flow to the equivalent level in the control, hydroxyfasudil-treated (0.1 mg kg^{-1}) and fasudil-treated (0.3 mg kg^{-1}) group. Although at 5 min after the initiation of pacing the increase in blood flow through LCX produced by hydroxyfasudil (0.3 mg kg^{-1}) and pacing was 155.0% of the baseline, the difference was not significant (Figure 3).

Blood flow profile

Hydroxyfasudil was administrated by continuous i.v. infusion (0.1 and 0.3 mg kg^{-1} for 30 min). Figure 4 shows the time courses of the changes in the coronary blood flow (CBF), vertebral blood flow (VBF) and femoral blood flow (FBF). Hydroxyfasudil dose-dependently increased CBF or VBF. The maximal increase in CBF or VBF produced by 0.3 mg kg^{-1} of hydroxyfasudil was 160.1 and 123.1% of the baseline, respectively, and occurred at 30 min after the start of i.v. administration. By contrast, hydroxyfasudil produced no significant increase in FBF (Figure 4).

Cardiac conduction system

Hydroxyfasudil (2 mg kg^{-1} for 20 min) decreased MBP with an increase in HR, and shortened the RR interval, but did not affect the QTc interval (Figure 5). Diltiazem (2 mg kg^{-1} for 20 min) decreased MBP and prolonged the PR interval. Fasudil (2 mg kg^{-1} for 20 min) and nicorandil (2 mg kg^{-1} for 20 min) decreased MBP and did not affect PR, RR or QTc intervals (Figure 5).

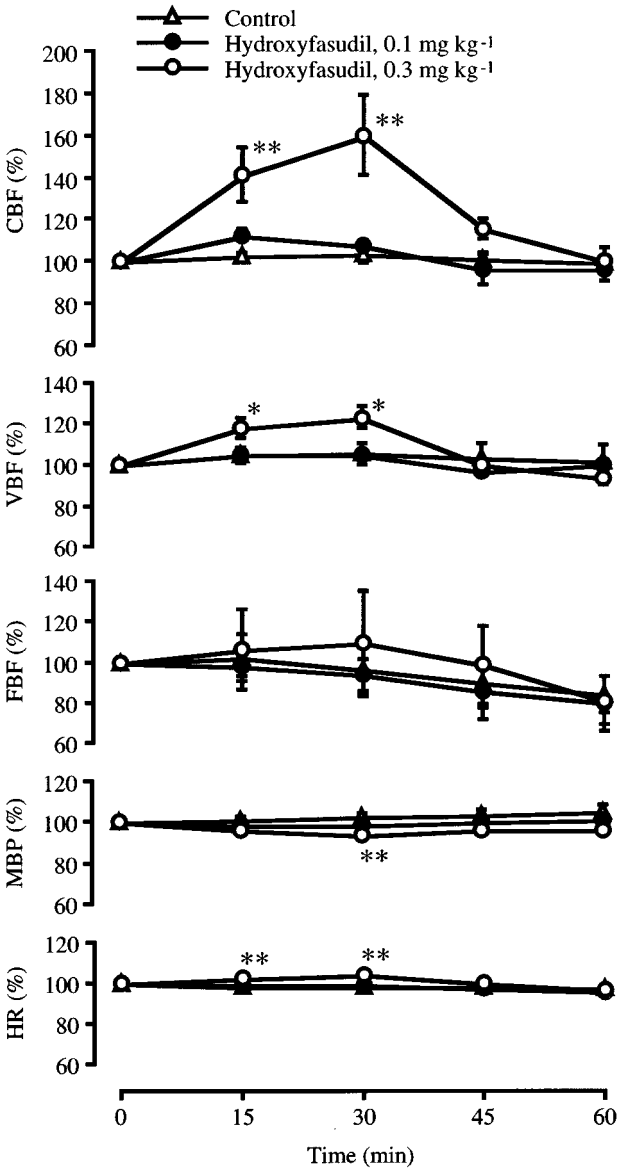

Figure 4 Change in blood flow, blood pressure and heart rate after continuous i.v. infusion of saline, hydroxyfasudil (0.1 mg kg^{-1} or 0.3 mg kg^{-1}) to anaesthetized dogs. Each data point represents the mean ± s.e.mean of six experiments. The asterisks indicate a significant difference from the control (**$P<0.01$, *$P<0.05$: Dunnett's test). Abbreviations: CBF, coronary blood flow; VBF, vertebral blood flow; FBF, femoral blood flow; MBP, mean blood pressure; HR, heart rate.

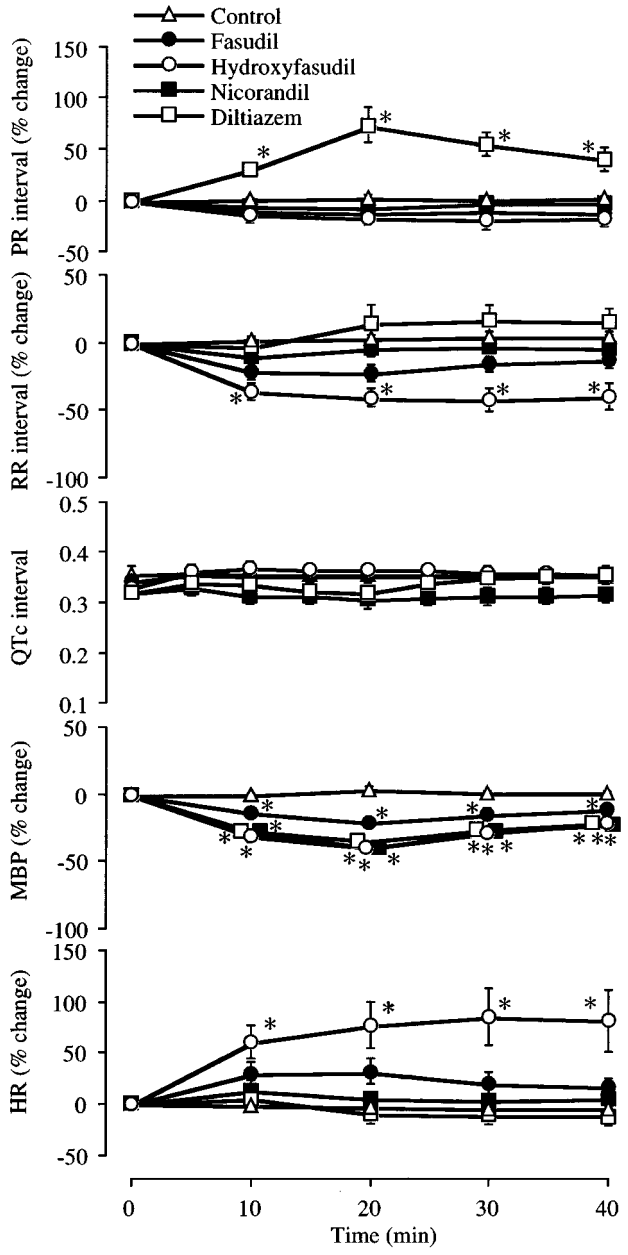

Figure 5 Effects of hydroxyfasudil, fasudil, nicorandil or diltiazem (2 mg kg^{-1}) on mean blood pressure (MBP), heart rate (HR) and cardiac conduction system in anaesthetized dogs. Each data point represents the mean ± s.e.mean of four experiments. The asterisks indicate a significant difference from the control (* $P<0.05$: Dunnett's test).

Isolated guinea-pig atria

Hydroxyfasudil (10^{-7}–10^{-4} M) showed no effect on the contractile force of the electrically paced left atria or the spontaneous beating rate of the right atria (Figure 6).

Discussion

There has been general agreement that an increased magnitude of ST-segment depression usually denotes an increased degree in the subendocardial ischaemia (Sugiyama & Hashimoto, 1999). In the present canine model of angina pectoris, RMBF decreased markedly in the LAD perfused endomyocardium region, and ST-segment depression appeared. Since oxygen consumption was increased to the maximum level by the tachy-pacing in the presence of LAD stenosis, an increase in oxygen supply to the ischaemic region or direct protection on the cardiomyocytes or both may be crucial to inhibit the myocardial cell damage. In the present study, hydroxyfasudil or fasudil appeared to protect myocardium subjected to pacing-induced ischaemia through the increase in RMBF. In dogs administered a low dose of

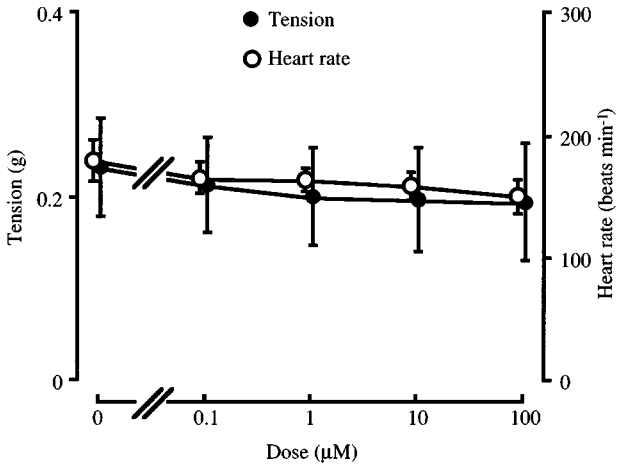

Figure 6 Lack of effect of increasing concentrations of hydroxyfasudil on the inotropic or chronotropic actions in the isolated guinea pig left or right atria. Each data point represents the mean ± s.e.mean of three experiments.

hydroxyfasudil (0.1 mg kg^{-1}) or fasudil (0.3 mg kg^{-1}), there were no significant changes in the blood flow through LAD or LCX before and during the pacing. A high dose of hydroxyfasudil (0.3 mg kg^{-1}) also did not affect the blood flow through LAD. These findings suggest that hydroxyfasudil or fasudil improved perfusion in the ischaemic zones supplied by collateral circulation. Nicorandil, a coronary vasodilator with a mechanism of potassium channel opening, is used clinically to treat angina pectoris. In the present study, nicorandil was shown to be effective and equipotent to fasudil in suppressing the ST-segment depression. Hydroxyfasudil was more potent in attenuating the ST-segment depression than was nicorandil or fasudil. Further evaluations, for example the comparison between hydroxyfasudil and other reference drugs used worldwide for the treatment of angina pectoris or the comparison between plasma concentrations and therapeutic efficacy, may help define the efficacy of hydroxyfasudil.

The anti-ischaemic effects of beta blockers and heart rate-lowering calcium blockers are the consequence of the reduction in the heart rate, inotropism and oxygen demand (Spaulding *et al*., 1997; Parmley, 1998), but attention should be paid to the possible additive negative inotropic or chronotropic effects of beta blockers and calcium blockers on the myocardium (Little *et al*., 1995; Sellier, 1996). Hence, despite a large number of anti-angina drugs, such as calcium blockers and beta blockers, there still remains a need for new drugs with enhanced efficacy and improved tolerability. New drugs should lack any negative inotropism, exhibit a high degree of vascular selectivity, and have a low incidence of side effects (Noll & Luscher, 1998).

We previously reported that higher concentrations (up to 3×10^{-4} M) of fasudil had no effects on inotropism of the guinea-pig heart *in vitro*, and this indicated that fasudil, unlike Ca^{2+} entry blockers, did not have a blocking action on slow myocardial Ca^{2+} channels (Asano *et al*., 1987). Hydroxyfasudil has also shown no inotropic or chronotropic effects in the isolated heart of guinea-pigs at concentrations up to 10^{-4} M. The plasma levels of hydroxyfasudil were not measured after intravenous infusion of hydroxyfasudil in dogs. In clinical trials, the maximum plasma concentration of fasudil and hydroxyfasudil were approximately $3-4 \times 10^{-7}$ M after an intravenous infusion of fasudil (30 mg for 30 min: approximately 0.5 mg kg^{-1} for 30 min). In the present study, larger concentrations, at least 100 fold the clinical plasma concentration, showed no inotropic or chronotropic effect. In the present studies, diltiazem significantly prolonged the PR interval in dogs, and this observation is in agreement with other experimental studies (Browne *et al*., 1983; Skarvan & Priebe, 1988). However, hydroxyfasudil did not affect the PR or QTc intervals at a high dose (2 mg kg^{-1} for 20 min), at least 6 to 20 fold higher than its effective doses (0.1 and 0.3 mg kg^{-1} for 30 min) in a canine model of effort angina. These findings suggest that hydroxyfasudil, unlike calcium entry blockers, does not act as a cardiodepressant by interfering with the cardiac slow Ca^{2+} channels, and hydroxyfasudil is well tolerated when used for the management of patients with left ventricular dysfunction or used with beta blockers or heart rate-lowering calcium blockers.

We previously reported that bolus intravenous administration of fasudil (0.01 – 0.3 mg kg^{-1}) dose-dependently increased VBF, CBF and FBF (Asano *et al*., 1989). In the present study, continuous intravenous infusion of hydroxyfasudil also significantly increased CBF or VBF. By contrast, hydroxyfasudil produced no significant increase in FBF. These findings indicate that hydroxyfasudil exhibit a vascular selectivity.

Shimokawa *et al*. (1999) reported that hydroxyfasudil potently inhibited Rho-kinase (IC_{50}, 0.9 – 1.8 μM), while its inhibitory effect was markedly (at least 50 – 100 times) less for myosin light chain kinase (MLCK) or protein kinase C (PKC), and these findings indicate that hydroxyfasudil preferentially inhibits Rho-kinase activity. A recent study confirmed that oral administration of fasudil prevented the occurrence of endothelin induced histological dysfunction in the myocardium (Yamamoto *et al*., 2000). The present and previous findings suggest that hydroxyfasudil and fasudil, as Rho-kinase inhibitors, are novel anti-angina drugs that have a potent vasodilator effect, increase blood supply to the ischaemic region of the myocardium and have a myocardial protective effect. To determine the efficacy of hydroxyfasudil for the prevention of myocardial injury, it may be useful to examine the effects of hydroxyfasudil on the indicators of myocardial cell damage, for example creatine kinase and troponin T, in the model of angina.

Inhibition of Rho-kinase appears to protect the myocardium subjected to pacing-induced ischaemia through the increase in the regional myocardial blood flow, and hydroxyfasudil may be categorized as a novel type of anti-anginal drug, without any inotropic and chronotropic effects.

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