Effects of KRN2391, nicorandil and diltiazem on the changes in the electrocardiogram caused by endothelin-1 in anaesthetized rats

¹Katsuhiko Harada, Atsushi Miwa, Soichiro Kaneta, Toshio Izawa, Hideaki Fukushima & Nobuyuki Ogawa

Pharmaceutical Research Laboratory, Kirin Brewery Co. Ltd., Miyahara-cho 3, Takasaki, Gunma 370-12 Japan

1 The effect of KRN2391, a novel vasodilator, on the changes of electrocardiogram caused by endothelin-1 (ET-1) was studied in anaesthetized rats and compared with the effects of nicorandil and diltiazem. In addition, the effect of KRN2391 on the action potential of guinea-pig papillary muscle was studied.

2 The intracoronary administration (i.c.) of ET-1 (5 µg) induced not only ST segment elevation of the electrocardiogram due to contraction of the coronary artery, but also arrhythmias involving atrio-ventricular block (A-V block), ventricular premature contraction (VPC) and ventricular fibrillation (VF), and resulted in death in most animals. However, the administration of methacholine (3 µg, i.c.) produced ST segment elevation alone without developing arrhythmias.

3 Pretreatment with intravenous administration of KRN2391 (30 µg kg⁻¹) inhibited the ST segment elevation and the development of arrhythmias induced by ET-1, and decreased the incidence of death.

4 Nicorandil (1000 µg kg⁻¹) prevented the ST segment elevation without suppression of the occurrence of VF. Diltiazem (100 µg kg⁻¹) suppressed both the ST segment elevation and the occurrence of VF but not other arrhythmias. Nicorandil at 3000 µg kg⁻¹ and diltiazem at 300 µg kg⁻¹ produced not only a suppression of ST segment elevation and VF incidence but also a decrease in the occurrence of arrhythmias. These doses of nicorandil and diltiazem produced a decrease in death in a dose-dependent manner.

5 KRN2391 (10 and 30 µg kg⁻¹), nicorandil (1000 and 3000 µg kg⁻¹) and diltiazem (100 and 300 µg kg⁻¹) significantly decreased mean blood pressure in a dose-dependent manner. Heart rate was decreased by nicorandil (3000 µg kg⁻¹) and diltiazem (100 and 300 µg kg⁻¹) but was not affected by KRN2391 (10 and 30 µg kg⁻¹).

6 KRN2391 (30 µM) significantly shortened the action potential duration of guinea-pig ventricle at 50% and 90% repolarization (APD₅₀ and APD₉₀). The effect of KRN2391 was inhibited by a K⁺ channel blocker, glibenclamide (30 µM).

7 These results suggest that the occurrence of ST segment elevation and arrhythmias induced by ET-1 are due to a dual direct action on both coronary vascular smooth muscle and myocardium. Therefore, the protective effects of KRN2391, nicorandil and diltiazem on ET-1-induced heart disorders appear to be due to their direct actions on coronary vascular smooth muscle and the myocardium.

Keywords: Endothelin; KRN2391; nicorandil; diltiazem; ST segment elevation; arrhythmia

Introduction

At present, coronary spasm is thought to be a cause of variant angina. Although many factors, such as endogenous vasoconstrictors derived from endothelial cells and platelets, have been demonstrated, the precise mechanism of coronary vasospasm remains unclear. Endothelin is now considered to play an important role in the development of hypertension and variant angina since endothelin was discovered as a novel potent vasoconstrictor released from endothelial cells (Yanagisawa *et al.*, 1988). Toyooka *et al.* (1991) observed clinically that the coronary sinus blood concentrations of endothelin increased in patients with variant angina and probable coronary vasospasm. In addition, when endothelin was administered into the coronary artery of dogs or rats, coronary constriction and ST segment elevation of the electrocardiogram similar to the clinical phenomenon of variant angina were observed (Nichols *et al.*, 1990; Yorikane & Koike, 1990).

Recently, we identified a novel vasodilator, KRN2391 (Figure 1), possessing both a K⁺ channel opening action

and a nitrate action (Kashiwabara *et al.*, 1991). KRN2391 may be useful in ischaemic heart disease because it shows selectivity for the coronary vascular beds in dogs (Ogawa *et al.*, 1990) and protective effects in vasopressin- (Harada *et al.*, 1990), isoprenaline- and methacholine-induced anginal models in rats (Harada *et al.*, 1991). In the present study, we have examined the effect of KRN2391 on the anginal characteristics induced by the intracoronary administration of endothelin-1 (ET-1) and compared it with those of other antianginal drugs, nicorandil and diltiazem, in anaesthetized rats.

Figure 1 Chemical structure of KRN2391.

¹ Author for correspondence.

Methods

Electrocardiogram measurements

Male Wistar rats weighing 250–310 g were anaesthetized with urethane and α -chloralose (1 g kg^{-1} , i.p. and 25 mg kg^{-1} , i.p., respectively). The change in the ST-segment of the Lead II electrocardiogram (ECG) was used to monitor ischaemia. A polyethylene cannula was introduced from the right carotid artery to inject ET-1 and its tip was positioned at the coronary ostia. The position of the tip of the cannula was confirmed by the elevation in ST segment induced by methacholine ($3 \mu\text{g}$, i.c.). It is well known that the intracoronary administration of methacholine causes an elevation of ST segment of the ECG by myocardial ischaemia related to coronary spasm (Aono *et al.*, 1981; Sakai *et al.*, 1981). ET-1 was injected over 5 s in a volume of $20 \mu\text{l}$. KRN2391, nicorandil and diltiazem were administered intravenously through the femoral vein, 2 min before the injection of ET-1. A Lead II ECG was recorded using an electrocardiograph (AP-621G, Nihon Kohden, Tokyo, Japan). The effects of KRN2391, nicorandil and diltiazem on ET-1-induced ECG changes were examined in different animals.

Blood pressure and heart rate measurements

In a separate group of animals (male Wistar rats weighing 270–310 g) blood pressure was measured via a cannula in the carotid artery using a pressure transducer (TP-200T, Nihon Kohden, Tokyo, Japan) through a carrier amplifier (AP-647G, Nihon Kohden, Tokyo, Japan). Mean blood pressure (MBP) was calculated as follows: $\text{MBP (mmHg)} = (\text{systolic blood pressure} - \text{diastolic blood pressure}) \times 1/3 + \text{diastolic blood pressure}$. Heart rate (HR) was measured by a heart rate counter (AT-601G, Nihon Kohden, Tokyo, Japan) triggered by the pulses. Drugs were administered into the femoral vein.

Action potential recording

Male guinea-pigs weighing 500–550 g were anaesthetized with sodium pentobarbitone (40 mg kg^{-1} , i.p.). The hearts

Figure 3 The effect of KRN2391 on ST segment elevation induced by the intracoronary administration of endothelin-1 (ET-1) in anaesthetized rats. (○) Responses in vehicle-treated animals ($n = 6$); (●) responses in KRN2391 ($10 \mu\text{g kg}^{-1}$)-treated animals ($n = 5$); (□) responses in KRN2391 ($30 \mu\text{g kg}^{-1}$)-treated animals ($n = 5$). Each point represents the mean $\pm$ s.e.mean. *Significant difference from the corresponding value in the vehicle-treated group ($P < 0.05$).

were rapidly excised and placed in Tyrode solution equilibrated with 95% O_2 and 5% CO_2 . Papillary muscles less than 1 mm in diameter and 2–3 mm in length were separated and mounted in a tissue chamber (3 ml volume). The preparation was superfused at a rate of 6 ml min^{-1} with Tyrode solution equilibrated with 95% O_2 and 5% CO_2 . The temperature of the tissue bath was maintained at $35 \pm 0.5^\circ\text{C}$. The preparations were stimulated electrically at a rate of 0.5 Hz with rectangular pulses of 1 ms duration and twice threshold voltage through bipolar platinum electrodes, and equilibrated for at least 60 min after excision. Membrane potentials were recorded via glass microelectrodes filled with 3 M KCl (resistances of 15–25 $\text{M}\Omega$) using a d.c. preamplifier (MEZ-8300, Nihon Kohden, Tokyo, Japan) and were displayed using a

Figure 2 Typical recording of the changes of electrocardiogram induced by intracoronary administration of methacholine (a) and endothelin-1 (ET-1) (b) in anaesthetized rats.

memory oscilloscope (VC-11, Nihon Kohden, Tokyo, Japan). Membrane potentials were recorded onto an electric recorder (WT-647G, Nihon Kohden, Tokyo, Japan). Action potentials were recorded with single cell impalements before and 15 min after exposure to each concentration of the drug applied cumulatively into the superfusate. Action potential parameters measured were the resting membrane potential (RP), the action potential amplitude (APA), and the action potential duration at the levels of 20, 50 and 90% repolarization (APD_{20} , APD_{50} , APD_{90}). The maximum rate of rise of the action potential (V_{max}) was obtained with an electronic differentiator (EQ-601G, Nihon Kohden, Tokyo, Japan).

Drugs and solutions

Tyrode solution for microelectrode studies contained (in mM): NaCl 137, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 1.8, NaH_2PO_4 11.9, glucose 5.5. KRN2391 and nicorandil were synthesized in our laboratory. Diltiazem hydrochloride, methacholine chloride and glibenclamide were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.) and ET-1 from the Peptide Institute (Osaka, Japan). KRN2391, diltiazem hydrochloride, nicorandil and methacholine chloride were

dissolved in saline for intravenous administration. For action potential measurements, KRN2391 was dissolved in distilled water. Glibenclamide (10 mM) was dissolved in dimethylsulphoxide. ET-1 was dissolved in saline and stored in a polyethylene vial at $-20^\circ C$ until used.

Statistical analysis

Differences were considered at a level of significance of $P < 0.05$, using Student's *t* test for paired data for single comparisons and analysis of variance for multiple comparisons. When multiple comparisons were made with a single control, Dunnett's test was used to determine levels of significance. Data are presented as the mean $\pm$ s.e.mean.

Results

Effects of intracoronary administration of methacholine and endothelin-1 on ECG

A typical record of the ECG observed following the administration of methacholine ($3 \mu g$, i.c.) or ET-1 ($5 \mu g$, i.c.) is shown in Figure 2. Methacholine induced transient cardiac arrest followed by ST segment elevation of the ECG. The duration of ST segment elevation by methacholine was less than 10 s. ET-1 also produced ST segment elevation and the duration of ST segment elevation by ET-1 was longer than that produced by methacholine. In addition, arrhythmias involving atrio-ventricular block (A-V block), ventricular premature contraction (VPC) and ventricular fibrillation (VF) were present 2.6 to 5.0 min after administration of ET-1. These arrhythmias resulted in death of four out of six rats in the control observations. However, methacholine did not induce arrhythmias and death, in spite of showing ST segment elevation.

Effects of KRN2391, nicorandil and diltiazem on endothelin-1 ($5 \mu g$, i.c.)-induced heart disorders

Pretreatment with KRN2391, $30 \mu g kg^{-1}$, i.v., significantly inhibited ST segment elevation induced by ET-1 ($5 \mu g$, i.c.) (Figure 3). A dose of $10 \mu g kg^{-1}$ KRN2391 also tended to attenuate ET-1-induced ST segment elevation but this effect was not significant. Although ET-1 caused arrhythmias in all rats and death in four out of six rats in the control group, arrhythmias and death in rats given KRN2391 at $30 \mu g kg^{-1}$ occurred in two out of five rats and one out of five rats, respectively. That is, the occurrences of A-V block, VPC and VF were decreased by $30 \mu g kg^{-1}$ KRN2391 (Figure 4).

Nicorandil at 1000 and $3000 \mu g kg^{-1}$ significantly suppressed the ST segment elevation induced by ET-1 to almost the same level as KRN2391 (Figure 5). Nicorandil at $1000 \mu g kg^{-1}$ did not decrease the occurrence of arrhythmias by ET-1, but decreased the incidence of death to two out of seven rats (Figure 6). Nicorandil at $3000 \mu g kg^{-1}$ diminished the occurrence of VF and death to one out of seven rats compared with four out of six rats given vehicle.

Diltiazem, at 100 and $300 \mu g kg^{-1}$, significantly inhibited ET-1-induced ST segment elevation to almost the same level (Figure 7). Diltiazem at $100 \mu g kg^{-1}$ decreased the occurrences of VF and death, i.e. these were observed in four out of six rats given vehicle but in two out of seven rats given $100 \mu g kg^{-1}$ (Figure 8). However, diltiazem ($100 \mu g kg^{-1}$) did not suppress arrhythmias with the exception of VF. A dose of $300 \mu g kg^{-1}$ diltiazem decreased the occurrence of arrhythmias involving VF.

Effects of KRN2391, nicorandil and diltiazem on haemodynamics

Table 1 shows the effects of KRN2391, nicorandil and diltiazem at the doses which were used to examine their effects

Figure 4 The effect of KRN2391 on arrhythmias induced by the intracoronary administration of endothelin-1 (ET-1) in anaesthetized rats. VF: ventricular fibrillation; VPC: ventricular premature contraction; A-V block: atrio-ventricular block.

Figure 5 The effect of nicorandil on ST segment elevation induced by intracoronary administration of endothelin-1 (ET-1) in anaesthetized rats. (○) Responses in vehicle-treated animals ($n = 7$); (●) responses in nicorandil ($1000 \mu g kg^{-1}$)-treated animals ($n = 7$); (□) responses in nicorandil ($3000 \mu g kg^{-1}$)-treated animals ($n = 7$). Each point represents the mean $\pm$ s.e.mean. *Significant difference from the corresponding value in the vehicle-treated group ($P < 0.05$).

Figure 6 The effect of nicorandil on arrhythmias induced by intracoronary administration of endothelin-1 (ET-1) in anaesthetized rats. VF: ventricular fibrillation; VPC: ventricular premature contraction; A-V block: atrio-ventricular block.

Figure 7 The effect of diltiazem on ST segment elevation induced by intracoronary administration of endothelin-1 (ET-1) in anaesthetized rats. (○) Responses in vehicle-treated animals ($n = 6$); (●) responses in diltiazem ($100 \mu\text{g kg}^{-1}$)-treated animals ($n = 7$); (□) responses in diltiazem ($300 \mu\text{g kg}^{-1}$)-treated animals ($n = 6$). Each point represents the mean $\pm$ s.e.mean. *Significant difference from the corresponding value in the vehicle-treated group ($P < 0.05$).

on ET-1-induced heart disorders, on MBP and HR. KRN2391 (10 and $30 \mu\text{g kg}^{-1}$), nicorandil (1000 and $3000 \mu\text{g kg}^{-1}$) and diltiazem (100 and $300 \mu\text{g kg}^{-1}$) all produced decreases in MBP in a dose-dependent manner. A decrease in HR was induced by nicorandil at $3000 \mu\text{g kg}^{-1}$ and diltiazem at 100 and $300 \mu\text{g kg}^{-1}$. KRN2391 at 10 and $30 \mu\text{g kg}^{-1}$ and nicorandil at $1000 \mu\text{g kg}^{-1}$ had no effect on HR. The effects of these drugs on MBP reached a peak 1–2 min after administration. Hypotensive effects of nicorandil lasted more than 60 min at both doses, those of KRN2391 at 10 and $30 \mu\text{g kg}^{-1}$ lasted for about 10 and 20 min, respectively, and those of diltiazem at 100 and $300 \mu\text{g kg}^{-1}$ lasted for about 15 and 40 min, respectively.

Electrophysiological effects of KRN2391

KRN2391 ($30 \mu\text{M}$) significantly shortened APD_{50} and APD_{90} of guinea-pig papillary muscles, but had no effect on other parameters (Table 2). The effect of KRN2391 ($30 \mu\text{M}$) on action potential duration was inhibited by glibenclamide ($30 \mu\text{M}$) (Table 2).

Figure 8 The effect of diltiazem on arrhythmias induced by intracoronary administration of endothelin-1 (ET-1) in anaesthetized rats. VF: ventricular fibrillation; VPC: ventricular premature contraction; A-V block: atrio-ventricular block.

Table 1 Effects of KRN2391, nicorandil and diltiazem on mean blood pressure and heart rate in anaesthetized rats

Drug ($\mu\text{g kg}^{-1}$)		MBP ($\Delta\%$)	HR ($\Delta\%$)
KRN2391	10	$-7.0 \pm 1.7^*$ (115 $\pm$ 5)	1.9 ± 1.6 (450 $\pm$ 20)
	30	$-22.9 \pm 3.3^*$ (111 $\pm$ 4)	3.5 ± 2.8 (454 $\pm$ 14)
Nicorandil	1000	$-34.9 \pm 3.9^*$ (113 $\pm$ 6)	0.9 ± 1.0 (441 $\pm$ 14)
	3000	$-52.4 \pm 2.8^*$ (117 $\pm$ 1)	$-22.7 \pm 1.9^*$ (468 $\pm$ 8)
Diltiazem	100	$-12.5 \pm 1.8^*$ (112 $\pm$ 8)	$-9.0 \pm 1.0^*$ (425 $\pm$ 9)
	300	$-26.0 \pm 2.4^*$ (112 $\pm$ 6)	$-21.8 \pm 1.7^*$ (428 $\pm$ 18)

MBP: mean blood pressure, HR: heart rate. The changes in MBP and HR are presented as the percent changes from the basal MBP (mmHg) and HR (beats min^{-1}) values (parentheses). Values are mean $\pm$ s.e.mean. ($n = 4$). *Significantly different from the basal values $P < 0.05$.

Discussion

The present results showed that ET-1 induced not only ST segment elevation due to myocardial ischaemia but also produced arrhythmias. In contrast, methacholine produced ST segment elevation without associated arrhythmias and death. The results with ET-1 and methacholine in the present studies were similar to those obtained in previous reports (Aono *et al.*, 1981; Sakai *et al.*, 1981; Yorikane & Koike, 1990). The effects of ET-1 and methacholine are thought to be due to myocardial ischaemia related to the contraction of coronary arteries. It has been observed that ET-1 and methacholine produce coronary vasoconstriction in isolated perfused hearts of rats (Aono *et al.*, 1981; Sakai *et al.*, 1981; Neubauer *et al.*, 1990). However, arrhythmias induced by ET-1 were not solely due to myocardial ischaemia, because methacholine did not produce arrhythmias in spite of ST segment elevation. Therefore, ET-1 may produce an increase in intracellular Ca^{2+} in the myocardium in addition to vasoconstriction, because increase in Ca^{2+} appears to play an important role in the development of ventricular arrhythmias (Billman, 1989). Lauer *et al.* (1989) reported that ET-1 increased intracellular Ca^{2+} in ventricular myocytes. Yorikane *et al.* (1991) also observed that ET-1 induced early afterdepolarizations contributing to the development of

Table 2 Effects of KRN2391 and glibenclamide (Glib) on action potential of guinea-pig papillary muscles

	APA (mV)	RP (mV)	APD ₂₀ (ms)	APD ₅₀ (ms)	APD ₉₀ (ms)	V _{max} (V s ⁻¹)
Control	139 ± 1	-96 ± 2	150 ± 17	241 ± 14	297 ± 11	141 ± 8
KRN2391 10 µM	138 ± 2	-99 ± 2	130 ± 16	214 ± 17	271 ± 15	142 ± 8
KRN2391 30 µM	130 ± 6	-98 ± 2	89 ± 19	140 ± 26*	181 ± 29*	128 ± 8
KRN2391 30 µM + Glib 30 µM	138 ± 2	-100 ± 3	141 ± 29	244 ± 21	299 ± 19	130 ± 8

APA: action potential amplitude, RP: resting membrane potential, APD₂₀, APD₅₀ and APD₉₀: action potential duration of 20, 50 and 90% repolarization, V_{max}: the maximum rate of rise of action potential. Values are mean ± s.e.mean. (n = 4).

*Significantly different from the values of control $P < 0.05$.

some types of arrhythmias. Thus, it is considered that the direct combined effects of ET-1 on coronary arteries and the myocardium contribute to the development of ST segment elevation and arrhythmias.

The present study showed that KRN2391 protected the heart from ST segment elevation and arrhythmias induced by ET-1. Nicorandil and diltiazem also suppressed ST segment elevation and arrhythmias induced by ET-1. However, the doses of nicorandil and diltiazem for the suppression of arrhythmia by ET-1 were higher than those required for suppression of ST segment elevation, because 1000 µg kg⁻¹ nicorandil and 100 µg kg⁻¹ diltiazem did not produce an obvious suppression of arrhythmia in spite of reducing ST segment elevation. The doses of KRN2391, nicorandil and diltiazem which produced suppression of the occurrence of arrhythmia were 30, 3000 and 300 µg kg⁻¹, respectively.

KRN2391 30 µg kg⁻¹ was the dose which produced a 22.9% decrease in MBP without affecting HR; 300 µg kg⁻¹ of diltiazem produced not only a 26.0% decrease in MBP but also a 21.8% decrease in HR. Nicorandil at 3000 µg kg⁻¹ also produced 52.4% and 22.7% reductions in MBP and HR, respectively. Thus, these results suggest that KRN2391 can suppress ET-1-induced arrhythmias at a dose which induced weak hypotension in comparison with nicorandil and diltiazem and did not affect HR.

The protective effects of KRN2391, nicorandil and diltiazem on ST segment elevation appear to be due to the inhibitory action on the contraction of coronary arteries induced by ET-1. Earlier studies suggested that ET-1-induced vasoconstriction was caused by the activation of voltage-dependent Ca²⁺ channels (Yanagisawa *et al.*, 1988) and the hydrolysis of phosphatidyl inositol which leads to Ca²⁺ release from the intracellular Ca²⁺ store (Van Renterghem *et al.*, 1988). Recently, Miyoshi *et al.* (1992) reported that ET-1 blocked ATP-sensitive K⁺ channels in the porcine coronary artery. In addition, ET-1-induced contraction of coronary arteries has been shown to be attenuated by Ca²⁺ channel blockers and K⁺ channel openers (Kim *et al.*, 1989; Turner *et al.*, 1989). Concerning the mechanism of vasodilatation induced by K⁺ channel openers, hyperpolarization caused by the increase in outward K⁺ current is thought to inhibit voltage-dependent Ca²⁺ channels (Cook *et al.*, 1988). Therefore, ET-1-induced vasoconstriction may be attenuated

by both Ca²⁺ channel blockers and K⁺ channel openers. Nicorandil, like KRN2391, show a dual mechanism of action as a nitrate and a K⁺ channel opener (Taira, 1987). Although nicorandil, like KRN2391, suppressed the ST segment elevation induced by ET-1, the antiarrhythmic effect of nicorandil was weaker than that of KRN2391. The mechanism for the effect of nicorandil on the coronary vascular beds and the myocardium may be different from that of KRN2391.

Recently, Yorikane *et al.* (1991) observed that ET-1 produced prolongation of action potential duration followed by the development of early afterdepolarizations in canine myocardial preparations, and suggested that the electrophysiological change in the myocardium caused by ET-1 was associated with the occurrence of arrhythmias. Furthermore, they reported that nicardipine, a Ca²⁺ channel blocker, inhibited the development of early afterdepolarizations caused by ET-1. This effect of nicardipine was thought to be based on a shortening of action potential duration, i.e. the prolongation of action potential duration by ET-1 was antagonized by nicardipine. In the myocardium, K⁺ channel openers, such as pinacidil and cromakalim, are also known to produce a shortening of action potential through an increase in K⁺ conductance (Osterrieder, 1988; Martin & Chinn, 1990). In addition, the shortening of action potential duration was also observed with nicorandil (Kojima & Ban, 1988). In the present study, KRN2391 produced a shortening of action potential duration through its K⁺ channel opening action. Therefore, it is considered that the effects of KRN2391, nicorandil and diltiazem on action potential contribute to the reduction of appearance of VF and death induced by ET-1.

In summary, we have shown that ET-1 induces arrhythmias in addition to ST segment elevation of the ECG and methacholine produces only ST segment elevation. The protective effects of KRN2391, nicorandil and diltiazem on ET-1-induced heart disorders are considered to be due to their direct actions on coronary vascular smooth muscle and on the myocardium. Although KRN2391 inhibited the occurrence of arrhythmia concomitantly with the suppression of ST segment elevation, the inhibitory effects of nicorandil and diltiazem on arrhythmias required higher doses than those needed to suppress ST segment elevation.

References

- AONO, J., AKIMA, M. & SAKAI, K. (1981). Effects of N-(2-hydroxyethyl)nicotinamide nitrate (SG-75) on methacholine-induced ECG changes in intact anesthetized rats. *Jpn. J. Pharmacol.*, **31**, 823–830.
- BILLMAN, G.E. (1991). The antiarrhythmic and antifibrillatory effects of calcium antagonists. *J. Cardiovasc. Pharmacol.*, **18** (Suppl. 10), S107–S117.
- COOK, N.S., WEIR, S.W. & DANZEISEN, M.C. (1988). Anti-vasoconstrictor effects of the K⁺ channel opener cromakalim on the rabbit aorta – comparison with the calcium antagonist isradipine. *Br. J. Pharmacol.*, **95**, 741–752.
- HARADA, K., FUKATA, Y., OGAWA, N. & NISHIKORI, K. (1990). Antianginal effect of KRN2391, a novel vasodilator, on the experimental model of angina pectoris induced by vasopressin treatment in anesthetized rats. *Jpn. J. Pharmacol.*, **52** (suppl. I), 216P.
- HARADA, K., FUKATA, Y., OHTA, H., OGAWA, N., FUKUSHIMA, H. & NISHIKORI, K. (1991). Antianginal effect of KRN2391 on the experimental models of anesthetized rats. *Jpn. J. Pharmacol.*, **55** (Suppl. I), 330P.

- KASHIWABARA, T., NAKAJIMA, S., IZAWA, T., FUKUSHIMA, H. & NISHIKORI, K. (1991). Characteristics of KRN2391, a novel vasodilator, compared with those of cromakalim, pinacidil and nifedipine in rat aorta. *Eur. J. Pharmacol.*, **196**, 1–7.
- KOJIMA, M. & BAN, T. (1988). Nicorandil shortens action potential duration and antagonises the reduction of $V_{\max}$ by lidocaine but not by disopyramide in guinea-pig papillary muscles. *Naunyn-Schmied. Arch. Pharmacol.*, **337**, 203–212.
- KIM, S., MORIMOTO, S., KOH, E., MIYASHITA, Y. & OGIHARA, T. (1989). Comparison of effects of a potassium channel opener BRL34915, a specific potassium ionophore valinomycin and calcium channel blockers on endothelin-induced vascular contraction. *Biochem. Biophys. Res. Commun.*, **164**, 1003–1008.
- LAUER, M.R., GUNN, M.D. & CIUSIN, W. (1989). Effect of endothelin on cytosolic calcium and membrane current in single ventricular myocytes. *Circulation*, **80** (Suppl. II), II–194.
- MARTIN, C.L. & CHINN, K. (1990). Pinacidil opens ATP-dependent K^+ channels in cardiac myocytes in an ATP- and temperature-dependent manner. *J. Cardiovasc. Pharmacol.*, **15**, 510–514.
- MIYOSHI, Y., NAKAYA, Y., WAKATSUKI, T., NAKAYA, S., FUJINO, K., SAITO, K. & INOUE, I. (1992). Endothelin blocks ATP-sensitive K^+ channels and depolarizes smooth muscle cells of porcine coronary artery. *Circ. Res.*, **70**, 612–616.
- NEUBAUER, S., ERTL, G., HAAS, U., PULZER, F. & KOCHSIEK, K. (1990). Effects of endothelin-1 in isolated perfused rat heart. *J. Cardiovasc. Pharmacol.*, **16**, 1–8.
- NICHOLS, A.J., KOSTER, P.F. & OHLSTEIN, E.H. (1990). The effect of diltiazem on the coronary haemodynamic and cardiac functional effects produced by intracoronary administration of endothelin-1 in the anaesthetized dog. *Br. J. Pharmacol.*, **99**, 597–601.
- OGAWA, N., KANETA, S., JINNO, Y., HARADA, K., OHTA, H., FUKUSHIMA, H. & NISHIKORI, K. (1990). Pharmacological profile of KRN2391, a novel vasodilator. *Eur. J. Pharmacol.*, **183**, 1266 (abstr.).
- OSTERRIEDER, W. (1988). Modification of K^+ conductance of heart cell membrane by BRL34915. *Naunyn-Schmied. Arch. Pharmacol.*, **337**, 93–97.
- SAKAI, K., AKIMA, M. & AONO, J. (1981). Evaluation of drug effects in a new experimental model of angina pectoris in the intact anesthetized rat. *J. Pharmacol. Methods*, **5**, 325–336.
- TAIRA, N. (1987). Similarity and dissimilarity in the mode and mechanism of action between nicorandil and classical nitrates: an overview. *J. Cardiovasc. Pharmacol.*, **10** (Suppl. 8), S1–S9.
- TOYO-OKA, T., AIZAWA, T., SUZUKI, N., HIRATA, Y., MIYAUCHI, T., SOO SHINN, W., YANAGISAWA, M., MASAKI, T. & SUGIMITO, T. (1991). Increased plasma level of endothelin-1 and coronary spasm induction in patients with vasospastic angina pectoris. *Circulation*, **83**, 476–483.
- TURNER, N.C., DOLLERY, C.T. & WILLIAMS, A.J. (1989). Endothelin-1-induced contractions of vascular and tracheal smooth muscle: effects of nicardipine and BRL 34915. *J. Cardiovasc. Pharmacol.*, **13** (Suppl. 5), S180–S182.
- VAN RENTERGHEM, C., VIGNE, P., BARHANIN, J., SCHMID-ALLIANA, A., FRELIN, C. & LAZDUNSKI, M. (1988). Molecular mechanism of action of the vasoconstrictor peptide endothelin. *Biochem. Biophys. Res. Commun.*, **157**, 977–985.
- YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332**, 411–415.
- YORIKANE, R. & KOIKE, H. (1990). The arrhythmogenic action of endothelin in rats. *Jpn. J. Pharmacol.*, **53**, 259–263.
- YORIKANE, R., KOIKE, H. & MIYAKE, S. (1991). Electrophysiological effects of endothelin-1 on canine myocardial cells. *J. Cardiovasc. Pharmacol.*, **17** (Suppl. 7), S159–S162.

(Received November 24, 1992

Revised March 3, 1993

Accepted March 4, 1993)