Effect of FK409, a Novel Nitric Oxide Donor, on Acute Experimental Myocardial Ischemia

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ABSTRACT—The anti-ischemic heart effect of (±)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (FK409), a novel nitric oxide donor, was studied in dog and rat preparations in vivo and in vitro. In anesthetized dogs with partially occluded coronary artery that were subjected to atrial pacing at a constant blood pressure, FK409 (1–100 pg/kg, i.v.) suppressed the ST-segment elevation on epicardial electrocardiograms. Glyceryl trinitrate (GTN; 10, 32 pg/kg) or dipyridamole (1000 pg/kg) failed to suppress the ST-segment elevation, although continuous i.v. infusion of GTN (32, 100 pg/kg/min) was effective. FK409 also suppressed the ST-segment elevation induced by methacholine in anesthetized rats by both i.v. (10, 100 pg/kg) and intraduodenal (i.d., 100, 100 pg/kg) injections, while GTN (100 pg/kg, i.v.; 1000 pg/kg, i.d.) was effective only by the i.v. route. FK409 (0.32 µg/kg/min, i.v.) and GTN (10 µg/kg/min) increased the blood flows of the endomyocardium (ENDO) and the epicardium (EPI) and the flow ratio of ENDO/EPI in the ischemic zone in anesthetized dogs with occluded coronary artery. Furthermore, in isolated dog vascular preparations, FK409 (4.6 x 10⁻¹⁰–4.6 x 10⁻⁷ M) had a greater vasorelaxing effect on the large coronary artery [2.0–2.5-mm outer diameter (od)] than on the small coronary artery (0.3–0.5-mm od) or the saphenous artery. The results suggest that FK409 protects against acute experimental myocardial ischemia through relaxation of the large conductive coronary artery, and may be a useful oral drug for the treatment of angina pectoris.

Keywords: FK409, Myocardial ischemia, Coronary artery, Vasodilation, Nitric oxide

(±)-(E)-4-Ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (FK409, Fig. 1) is a novel semisynthetic fermentation product of Streptomyces griseosporeus with vasodilating and anti-platelet activities (1). The compound exerted a potent relaxant effect on dog and rabbit isolated arteries contracted with U46619, PGF$_2$α, KCl, norepinephrine, histamine or 5-hydroxytryptamine (2–5); and its relaxant effect was, like that of glyceryl trinitrate (GTN) (6, 7), mediated through elevation of guanosine 3',5'-cyclic monophosphate (cyclic GMP) levels brought about by activation of soluble guanylate cyclase through nitric oxide formed from the FK409 molecule (2, 8). Furthermore, FK409 elicited less self-tolerance to its relaxant effect when compared with GTN, and it had relatively little cross-tolerance to organic nitrates (2, 5).

Thus, FK409 can be characterized as a unique vasodilator with nitric oxide-donating ability, and its ultimate therapeutic utility will likely be in the treatment of myocardial ischemic diseases. We therefore carried out the present study to define its effect on acute ischemic heart models.

Fig. 1. Chemical structure of FK409.
MATERIALS AND METHODS

Myocardial ischemia caused by partial occlusion of the coronary artery and atrial pacing in anesthetized dogs

The preparation was similar to that described by Szekeres et al. (9) (Fig. 2). Mongrel dogs of either sex, weighing 8.4 to 15.8 kg, were anesthetized with pentobarbital sodium (35 mg/kg, i.p.), and the thorax was opened under artificial respiration with room air (15–20 breaths/min). The left anterior descending coronary artery (LAD) was dissected at the origin, and a silk ligature was placed loosely around it for partial occlusion of the LAD. A flowmeter probe was also placed distal to the ligature so that no branches were present between the ligature and probe. For pacing of the heart, a bipolar silver electrode was clipped to the right auricular appendage. The parameters of the driving stimulus were as follows: duration 2 msec, voltage 1–3 V.

The myocardial ischemic changes were determined by direct monopolar epicardial leads (spiral-type, Hongo Seiki, Tokyo) with the left hind limb as the reference point. To identify the site of greatest ischemic change, three electrodes were applied simultaneously to the surface of the left anterior ventricular wall, and the electrode showing the greatest ST-segment elevation was used for evaluation (Fig. 2). Blood pressure (BP) was measured with a pressure transducer in the femoral artery, and heart rate (HR) was measured by a tachometer triggered by BP waves. All the parameters, including the epicardial electrocardiogram (ECG), were recorded on a polygraph (RM-85, Nihon Kohden, Tokyo).

The experimental procedure was as follows: After completing the preparation, about 60 min were allowed for stabilization. Then the LAD was partially occluded to

![Diagram of experimental method](https://example.com/diagram.png)

**Fig. 2.** Schematic diagram of the experimental method used to measure epicardial ST-segment elevation during atrial pacing in anesthetized dogs with partially occluded coronary artery. The heart was driven for 5 min at a rate 100/min higher than the basic rate 30 to 60 min after the partial coronary occlusion. The shaded area represents the ischemic region. LAD: left anterior descending artery.
reduce the coronary blood flow by two-thirds of the basal flow. After the partial occlusion, a 30- to 60-min stabilization period followed. The heart was then driven for 5 min at a rate 100/min higher than the basic rate to obtain ischemic ECG changes brought about by a pacing-induced work load (Fig. 2). The test compound was given i.v. together with small doses of phentolamine, an α-adrenoceptor stimulant, as necessary (2–4 μg/kg and/or 2–4 μg/kg/min) to maintain systemic BP at the pretreatment level.

Regional myocardial blood flow in anesthetized dogs with coronary artery occlusion

The study was performed according to the colored microsphere method described by Hale et al. (10). Beagle dogs of either sex, weighing 9.0 to 12.5 kg, were anesthetized with sodium pentobarbital (35 mg/kg, i.v.) and mechanically ventilated with room air; the tidal volume was 15 ml/kg and the rate was 15/min. The chest was then opened at the fifth left intercostal space, and the pericardium was incised. Catheters were introduced into the right femoral vein, left femoral artery and right femoral artery for drug administration, measurement of systemic BP and withdrawal of reference blood samples, respectively. Another catheter was inserted into the left atrium through the left atrial appendage for administration of microspheres. A pressure transducer-tipped catheter (PC380, Millar, Houston, TX, USA) was introduced into the left ventricle through the carotid artery for measurement of the maximum rate of rise of left ventricular pressure (LV max dp/dt). The LAD was isolated proximal to the first diagonal branch for later occlusion. HR, BP and LV max dp/dt were monitored throughout the duration of the experiment.

The following experimental procedure was used: Thirty minutes after occlusion of the LAD, the test compound was administered i.v. at the rate of 0.05 ml/kg/min for 30 min. Blood flow was measured with three types of colored polystyrene microspheres (12 μm, 5 × 10⁶ of each; E-Z Trac, Los Angeles, CA, USA) before and at 3 and 28 min after starting drug administration. A reference blood sample was obtained at the rate of 10 ml/min starting 10 sec before the injection of microspheres and continuing until 80 sec after injection (total collection time, 90 sec). After completion of flow measurement, the heart was removed and catheters were inserted into the LAD distal to the site of occlusion and into the aorta above the coronary ostia with the aortic valves closed. Then, the aorta and the LAD were respectively perfused with 0.25% Evans blue and saline under a constant pressure of 100 mmHg to delineate the in vivo risk zone of ischemia. The heart was excised; tissue samples (2–5 g each) from the unstained portion (i.e., total area at risk) and the stained portion (i.e., area-not-at-risk) of the left ventricle were obtained from each dog. Processing of tissue and blood samples to quantify colored microspheres followed the method described by Hale et al. (10). Regional myocardial blood flow (RMBF, ml/min/g) was computed with the formula: RMBF = (CT × R)/(CR × WT), where CT is the total number of microspheres in the tissue sample, R is reference flow rate (10 ml/min), CR is the total number of microspheres in the reference blood sample, and WT is weight of the tissue sample in grams.

Myocardial ischemia caused by intra-aortic injection of methacholine in anesthetized rats

The method was essentially the same as that described by Sakai et al. (11). Male Sprague-Dawley rats weighing 338 to 425 g were anesthetized with ethyl carbamate (1.25 g/kg, s.c.) and then intubated. For selective bolus injections of methacholine into the ostia of the coronary arteries, a polyethylene cannula was introduced through the exposed right carotid artery to a point near the aortic valve. Systemic BP and HR were measured via a pressure transducer (MPU-0.5, Nihon Kohden) in the femoral artery and a tachometer (AT601G, Nihon Kohden) triggered by BP waves respectively, and recorded on a Nihon Kohden WT-685G recorder. The standard limb lead II of the ECG was recorded by means of an electrocardiograph (ZS-501, Fukuda ME, Tokyo). The experimental procedure was as follows: After completing the preparation, about 20 min were allowed for stabilization. Then methacholine in doses of 4 to 8 μg in a volume of 0.01 ml were injected repeatedly into the aorta at intervals of 30 min. When reproducible ischemic ECG changes, i.e., ST-segment elevations, were obtained after injections of methacholine, the test compound was given i.v. 1 min or intraduodenally (i.d.) 5 min before the next dosing with methacholine.

Isolated dog and rabbit vascular preparations

Mongrel dogs of either sex, weighing 7 to 18 kg, were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and bled to death from the femoral artery to obtain the material for the vascular preparations. For the coronary artery, left circumflex artery (LCX, 2.0–2.5-mm outer diameter (od); large vessels) and small branches of the LAD (0.3–0.5-mm od, small vessels) were dissected from the myocardium and cut into spiral strips 10–15 mm by 0.5–1.0 mm for the large vessels and 5–7 mm by 0.2–0.5 mm for the small vessels. The saphenous arteries (1.0–1.5-mm od) were also removed and cut into spiral strips 10–15 mm in length. The functional integrity of the endothelium was not routinely checked because FK409 as well as GTN and adenosine are known to act directly on the smooth muscle cells (3, 12, 13). In separate
studies with the coronary or saphenous arteries, however, the involvement of endothelium was investigated by exposing strips precontracted with PGF$_{2\alpha}$ (2.8 $\mu$M) to acetylcholine (ACh, 1 $\mu$M). In these cases, all preparations ($n=5$ each) had over 60% relaxations after application of ACh, suggesting that they contained a viable endothelium (14).

Each strip was suspended in an organ bath containing a nutrient solution at 37 $^\circ$C and gassed with 95% O$_2$ and 5% CO$_2$. The tension of the strips was measured isometrically with a force-displacement transducer connected to a polygraph (RMP-6008, Nihon Kohden). The resting tension was adjusted to 60–100 mg for the small LAD and 1.0–1.5 g for the other arteries. Tyrode’s solution (136.9 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl$_2$, 1.0 mM MgCl$_2$, 11.9 mM NaHCO$_3$, 0.4 mM NaH$_2$PO$_4$ and 5.6 mM dextrose) was used for the coronary preparations, and Locke’s solution (154.0 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl$_2$, 6.0 mM NaHCO$_3$ and 5.6 mM dextrose) was used for the saphenous preparations. At least 1 hr was allowed for equilibration, after which the arterial strips were contracted by KCl, 9,11-azo-PGH$_2$ (azo-PGH$_2$) or PGF$_{2\alpha}$, in a concentration that caused a submaximal response (30–60 mM for KCl, 2.9–29 nM for azo-PGH$_2$, 2.8 $\mu$M for PGF$_{2\alpha}$). When the response to the agonist plateaued, cumulative concentrations of FK409 or other drugs were added; finally, a relaxant effect was obtained by treating each strip with a high concentration (0.1 mM) of papaverine. The responses to the test compound were expressed as percent of the maximum relaxation caused by papaverine.

Drugs

FK409 (Fujisawa, Osaka), papaverine hydrochloride (Nacalai Tesque, Kyoto), (−)-norepinephrine hydrochloride (Sigma, St. Louis, MO, USA) and methacholine chloride (Wako Junyaku, Osaka) were dissolved in deionized water or saline before use. GTN (Nihon Kayaku, Tokyo) dissolved in ethyl alcohol (EtOH) (10 mg/ml) and dipyridamole injection (Persantin, Nippon Boehringer Ingelheim, Kawanishi) were diluted with deionized water or saline. PGF$_{2\alpha}$ (Funakoshi, Tokyo) was dissolved in EtOH and then diluted with deionized water.

Statistical evaluation

The results were expressed as the mean±S.E.M. Statistical analyses were performed by Dunnett’s multiple test for more than two-group comparison. Differences in the mean values between two groups, or the predrug and postdrug values, were assessed with Student’s unpaired or paired $t$-test. Differences at the levels of $P<0.05$ were considered to be significant. The EC$_{50}$, the drug concentration required to produce 50% of the maximal papaverine-induced relaxation, was calculated according to the method of Litchfield and Wilcoxon.

RESULTS

Effect of FK409 on epicardial ST-segment elevation in anesthetized dogs with partial occlusion of the coronary artery and subsequent atrial pacing

As shown in Fig. 2, elevation of the ST-segment appeared within 2 min after pacing was started. This change increased with time, and ECG returned to the control value shortly after pacing was stopped. Therefore, pacing was maintained for 5 min, when marked ST-segment elevation had developed, and full recovery occurred during the subsequent 5 min at normal HR. The ECG change was entirely reproducible over an experimental period of approximately 90 min, when the 5-min atrial pacing was repeated at intervals of 30 min (Fig. 3, left).

FK409 in i.v.-doses of 1 to 100 $\mu$g/kg dose-dependently suppressed the elevation of the ST-segment under the systemic BP-controlled condition (Fig. 3, right; Table 1). In this case, increasing doses of the compound were injected at intervals of 30 min. On the other hand, GTN failed to suppress the ST-segment elevation in i.v.-doses of 10 and 32 $\mu$g/kg, although by continuous i.v.-infusion of 32 $\mu$g/kg/min or higher, it was effective, suggesting that GTN has a short-lived activity (Table 1). Dipyridamole (1000 $\mu$g/kg, i.v.), a coronary arteriolar segment dilator, did not improve the change of the ST-segment. Phenylephrine (4 $\mu$g/kg+4 pg/kg/min, i.v.) also did not have any effect on the ST-segment elevation (not shown in Table 1).

Effect of FK409 on ischemic regional myocardial blood flow in anesthetized dogs

As was the case with the control (see Table 2), regional myocardial blood flow (RMBF) in the EPI and the ENDO were reduced by LAD occlusion (LV normal zone vs. LV ischemic zone). The decrease in RMBF was greater in the ENDO than in the EPI region.

In this microspheres study, FK409 was continuously injected into the vein at a constant rate (see Methods) to obtain a stable blood level of the compound during the time for collection of reference blood. The dose of 0.32 $\mu$g/kg/min of FK409 was selected as a suitable infusion dose to obtain a plasma concentration of around 3 ng/ml, which was the effective plasma level of the compound in ischemic myocardial dogs with partially occluded coronary artery and subsequent atrial pacing after an i.v. bolus injection of FK409 at the dose of 10 $\mu$g/kg (average value at 5 to 10 min after injection).

FK409 at 0.32 $\mu$g/kg/min produced significant increases in the blood flow of the ENDO and EPI, and it
also increased the ratio of ENDO/EPI in the ischemic zone at both the 3- and 28-min time points (Table 2). On the other hand, there were no significant changes in any RMBF of the normal zone (Table 2) or in other cardiovascular parameters including BP (Table 3) during FK409 infusion. GTN at 10 μg/kg/min had a similar effect (Table 2), but 32 μg/kg/min caused severe hypotension (mean BP: −18 ± 1 mmHg at 5 min after starting the i.v.-infusion, n = 3), and its effect could not be evaluated.

Fig. 3. Effect of FK409 on pacing-induced epicardial ST-segment elevation in anesthetized dogs with partially occluded coronary artery. All values are the means ± S.E.M. of 3 to 5 experiments. Increasing doses of FK409 were given into the femoral vein at intervals of 30 min, together with small doses of phenylephrine (2–4 μg/kg and/or 2–4 μg/kg/min, i.v.) to maintain systemic BP at the pretreatment level. *P < 0.05, **P < 0.01 vs. value for control.

Table 1. Effect of FK409, GTN and dipyridamole on atrial pacing-induced epicardial ST-segment elevation in anesthetized dogs with partially occluded coronary artery

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (i.v.)</th>
<th>Maximum elevation of the ST-segment (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>predosing (control)</td>
</tr>
<tr>
<td>FK409</td>
<td>1 μg/kg</td>
<td>9.4 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>10 μg/kg</td>
<td>9.4 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>100 μg/kg</td>
<td>9.4 ± 1.1</td>
</tr>
<tr>
<td>GTN</td>
<td>10 μg/kg</td>
<td>7.7 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>32 μg/kg</td>
<td>7.7 ± 1.7</td>
</tr>
<tr>
<td>GTN</td>
<td>32 μg/kg/min</td>
<td>7.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.8 ± 0.8)</td>
</tr>
<tr>
<td></td>
<td>100 μg/kg</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>1000 μg/kg</td>
<td>7.3 ± 1.6</td>
</tr>
</tbody>
</table>

The test compound was given i.v. together with small doses of phenylephrine, an α-adrenoceptor stimulant, to animals as necessary (2–4 μg/kg and/or 2–4 μg/kg/min) to maintain BP at the control level. Phenylephrine in doses of 2–4 μg/kg or 2–4 μg/kg/min did not have any significant effect. Values are the mean ± S.E.M. of 3–5 experiments. *P < 0.05 vs. value for control. GTN, Glyceryl trinitrate. a the value at 5 min after starting the pacing. b 2 min after i.v. bolus administration or 5 min after starting the i.v. infusion. c increasing doses. d i.v. infusion for 10 min (μg/kg/min). e the sub-maximal elevation at 3 min after starting the pacing.
Table 2. Effect of intravenous FK409 and GTN on regional myocardial blood flow in normal and ischemic zones during acute coronary occlusion

<table>
<thead>
<tr>
<th>Region</th>
<th>3 min after starting compound i.v. infusion</th>
<th>28 min after starting compound i.v. infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (saline)</td>
<td>FK409 (0.32 pg/kg/min)</td>
</tr>
<tr>
<td>LV normal zone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENDO (ml/g/min)</td>
<td>0.85±0.11</td>
<td>0.88±0.08</td>
</tr>
<tr>
<td>EPI (ml/g/min)</td>
<td>0.86±0.13</td>
<td>0.86±0.11</td>
</tr>
<tr>
<td>ENDO/EPI</td>
<td>1.01±0.05</td>
<td>1.17±0.30</td>
</tr>
<tr>
<td>LV ischemic zone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENDO (ml/g/min)</td>
<td>0.12±0.01</td>
<td>0.22±0.01**</td>
</tr>
<tr>
<td>EPI (ml/g/min)</td>
<td>0.25±0.02</td>
<td>0.35±0.03*</td>
</tr>
<tr>
<td>ENDO/EPI</td>
<td>0.48±0.02</td>
<td>0.66±0.05**</td>
</tr>
</tbody>
</table>

The blood flow was measured with the colored microsphere method (10). Intravenous infusion of FK409 or GTN was started 30 min after LAD occlusion. Values are the mean±S.E.M. of 4–5 experiments. GTN, Glyceril trinitrate; LV, left ventricle; ENDO, endocardium; EPI, epicardium; ENDO/EPI, endocardial-to-epicardial blood flow ratio. *P<0.05, **P<0.01 vs. value for control.

Table 3. Effects of intravenous FK409 and GTN on mean blood pressure, heart rate and the maximum rate of rise of left ventricular pressure (LV max dp/dt) during acute coronary occlusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3 min after starting compound i.v. infusion</th>
<th>28 min after starting compound i.v. infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (saline)</td>
<td>FK409 (0.32 pg/kg/min)</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>89±13</td>
<td>89±17</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>126±12</td>
<td>106±9</td>
</tr>
<tr>
<td>LV max dp/dt (mmHg/sec)</td>
<td>1644±278</td>
<td>1480±201</td>
</tr>
</tbody>
</table>

Intravenous infusion with FK409 or GTN was started 30 min after LAD occlusion. Values are the mean±S.E.M. of 4–5 experiments. GTN, Glyceril trinitrate; LV, left ventricle. There were no significant changes in the parameters between the control and FK409 or GTN (P > 0.05).

Effect of FK409 on ST-segment elevation caused by intra-aortic injection of methacholine in anesthetized rats

Methacholine in intra-aortic doses of 4 to 8 µg evoked a transient hypotension, followed thereafter by marked elevation of the ST-segment and the T wave of the ECG, in accordance with the previous findings (11, 15). These ECG changes lasted 10 to 30 sec and were reproducible 4 to 6 times at injection intervals of 30 min.

FK409 in i.v.-doses of 10 and 100 µg/kg suppressed the elevation of the ST-segment (P<0.01 or 0.05; Fig. 4, upper graph). GTN at 100 µg/kg also was effective, and its % inhibition was almost comparable to that of FK409 at 10 µg/kg (41% vs. 42%). On the other hand, i.d. administration of FK409 was effective in doses of more than 100 µg/kg, about 10 times the i.v. effective dose, whereas GTN had no effect in an i.d. dose of 1000 µg/kg (Fig. 4, lower graph). These results indicate that FK409 has a more potent suppressing effect than GTN and exerts a therapeutic effect after absorption from the intestinal tract.

Effect of FK409 on isolated dog coronary and saphenous arteries

As shown in Fig. 5, FK409 in concentrations of 4.6 × 10⁻¹⁰ M and higher had dose-dependent relaxant effects on KCl- or azo-PGH₂, a stable thromboxane A₂ agonist (16),-induced contractions of the coronary (large vessel, 2.0–2.5-mm od) and saphenous arteries. The vasorelaxing effect of FK409, however, differed in the two arteries; the action of the compound was greater on the coronary artery than on the saphenous artery, with approximately a 150-fold difference between their EC₅₀ values: KCl-contracture, (6.2±1.3)×10⁻⁹ M for coronary artery vs. (9.0±5.7)×10⁻⁷ M for saphenous artery; azo-PGH₂-contracture, (3.9±0.9)×10⁻⁹ M for coronary artery vs. (5.9±2.4)×10⁻⁷ M for saphenous artery.

Similar results were observed in isolated dog coronary and saphenous arteries contracted with PGF₂α (not shown in Fig. 5): EC₅₀ values, (3.2±0.3)×10⁻⁹ M for coronary artery vs. (3.1±0.6)×10⁻⁸ M for saphenous artery, P<0.01, n=6 each.
Fig. 4. Effects of FK409 and glyceryl trinitrate (GTN) on methacholine-induced ST-segment elevation in anesthetized rats. All values are the means±S.E.M. of 6 to 12 experiments. The test compounds were given i.v. 1 min or intraduodenally (i.d.) 5 min before dosing with methacholine. *P<0.05, **P<0.01 vs. value for predosing.

Fig. 5. Relaxation caused by FK409 in KCl or azo-PGH₂-induced contractions of dog coronary and saphenous arteries. Relaxation at 0.1 mM papaverine was taken as 100%. Means±S.E.M. are presented. Figures in parentheses indicate the number of preparations used. **P<0.01, ***P<0.001 vs. value for the saphenous artery.
Table 4. Vasorelaxing effect of FK409 and GTN in isolated dog coronary (large vessel) arterial preparations

<table>
<thead>
<tr>
<th>Agonist</th>
<th>FK409</th>
<th>GTN</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 mM KCl</td>
<td>( (6.2 \pm 1.3) \times 10^{-9} )</td>
<td>( (1.6 \pm 0.5) \times 10^{-7} )</td>
</tr>
<tr>
<td>29 mM azo-PGH₂</td>
<td>( (3.9 \pm 0.9) \times 10^{-9} )</td>
<td>( (2.5 \pm 0.3) \times 10^{-4} )</td>
</tr>
<tr>
<td>2.8 ( \mu )M PGF₂₀</td>
<td>( (3.2 \pm 0.3) \times 10^{-9} )</td>
<td>( (1.6 \pm 0.5) \times 10^{-8} )</td>
</tr>
</tbody>
</table>

Values are the means±S.E.M. of 6 experiments. GTN, Glyceryl trinitrate. \(^*P<0.05, \quad **P<0.001\) vs. value for GTN. \( [ ] \): potency relative to that for FK409. Large vessel: left circumflex artery with 2.0–2.5-mm outer diameter.

The coronary vasorelaxing activities of FK409 were 5 to 26 times more effective than those of GTN (Table 4).

Comparative effect of FK409 on isolated dog large and small coronary arteries

The coronary vasorelaxing activity of FK409 was additionally examined in the vessels with small (0.3–0.5-mm od) and large (2.0–2.5-mm od) diameters by the method of Schnaar and Sparks (17). Since the responses of large and small vessels to a compound could not be directly compared because the small vessel, having less cross-sectional area, could not exhibit absolute tension changes as great as those of the large vessel, they were expressed as a percent of vessel relaxation after treatment with papaverine hydrochloride.

As shown in Fig. 6, FK409 had a more potent effect on the large vessels than on the small ones of the coronary arteries contracted by KCl. The \( EC_{50} \) values of the compound for the large and small arteries were \( (6.2 \pm 1.3) \times 10^{-9} \) M and \( (2.0 \pm 0.7) \times 10^{-7} \) M, respectively, with a difference of approximately 30-fold between the values \( (P<0.05) \). GTN also had a selective activity for the large vessels: \( EC_{50} \), \( (1.6 \pm 0.5) \times 10^{-7} \) M for the large artery, \( (1.8 \pm 0.6) \times 10^{-6} \) M for the small artery; \( EC_{50} \) ratio of small/large, 11; \( n=6 \) each. In contrast, adenosine was rather more active on the small artery: \( EC_{50} \), \( >3.7 \times 10^{-4} \) M for the large artery, \( (1.1 \pm 0.4) \times 10^{-4} \) M for the small artery; ratio, <0.3; \( n=6 \) each; Fig. 6.

A similar large vessel-selective effect of FK409 was observed with coronary arteries contracted by either azo-PGH₂ (not shown in Fig. 6): \( EC_{50} \), \( (3.9 \pm 0.9) \times 10^{-9} \) M for the large artery, \( (2.4 \pm 1.4) \times 10^{-8} \) M for the small artery, \( n=6 \) each or PGF₂₀: \( EC_{50} \), \( (3.2 \pm 0.3) \times 10^{-9} \) M for the large artery, \( (1.3 \pm 0.3) \times 10^{-8} \) M for the small artery, \( n=6 \) each, \( P<0.01 \).

DISCUSSION

The present findings with ischemic heart models suggest that FK409 will produce a therapeutic effect on ischemic heart diseases; i.e., the compound in i.v. bolus doses of 1 to 100 \( \mu \)g/kg was effective in suppressing epicardial ST-segment elevations in anesthetized dogs with partial occlusion of the coronary artery and subsequent atrial pacing. In this experiment, BP was controlled at a constant level to avoid disturbance of the coronary action of the compound due to hypotension. This ischemic heart model mimics, in certain aspects at least, the alterations in myocardial oxygen demand and supply in classical angina pectoris, and it has been reported to be useful for the evaluation of potential antianginal drugs (9, 18). Using a similar model, Szekeres et al. (9) found that a 10-min i.v.
infusion of GTN at 20 μg/kg reduced the epicardial ST-segment and T-wave elevations, whereas dipyridamole, a potent coronary arteriolar segment dilator, at 60 μg/kg failed to do this. Our findings for GTN and dipyridamole (Table 1) were in good agreement with those of Szekeres et al. In addition, FK409 was effective in suppressing the elevation of the ST-segment induced by methacholine in anesthetized rats (Fig. 4). This suppressing effect cannot be attributed to an antagonistic action toward vascular muscarinic receptors, because the compound has no anticholinergic activity (M. Ohtsuka et al., unpublished data). Sakai et al. (11) first reported that a single dose of methacholine, a cholinomimetic drug, administered intravenously evokes a marked elevation of the ST-segment and T-wave of the ECG in intact anesthetized rats, and suggested that the ECG changes induced by methacholine can be ascribed to coronary vasoconstriction, indicating severe myocardial anoxia or ischemia. This phenomenon in rats resembles the clinical sequelae of a certain type of angina pectoris (19, 20), and it was also reported that cholinomimetic drugs such as methacholine or pilocarpine are capable of producing a coronary arterial spasm corresponding to the ST-segment elevation in patients with variant angina (21).

It is noteworthy that FK409 was intraduodenally active, whereas this was not the case with GTN. The difference in their suppressing effects on methacholine-induced ECG changes after i.d.-administration is probably due to a difference in their absorption from the intestinal tract or in their first-pass extraction in animals, as the doses of FK409 and GTN we used were based on their in vitro vasorelaxing activities (2–5). GTN is well known to be degraded during its first pass through the liver. According to the present studies on vascular muscle preparations, the anti-ischemic heart effect of FK409 is highly likely to be due to its distinctive coronary vasorelaxant action. In our in vitro studies on isolated dog vascular strips, FK409 had a greater vasorelaxing activity on the coronary artery than on the saphenous artery; and moreover, its coronary activity was greater on the large vessels (2.0–2.5-mm od) than on the small ones (0.3–0.5-mm od). This effect was entirely the opposite of that of adenosine, a putative coronary metabolic vasodilator. The large coronary-selectivity of FK409 was rather superior to that of GTN, and its potency of activity was 5 to 26 times greater (Table 4). Fam and McGregor (22) and Winbury et al. (23) have demonstrated that GTN acts preferentially on the large coronary arteries in in situ studies with dogs and suggested that this results in increased blood flow to the ischemic region of the myocardium, and may be related to the anti-anginal effect of the drug. Schnaar and Sparks (17) further studied this possibility by examining the relaxing effect of GTN on the isolated vascular smooth muscle from large (2-mm od) and small (550-μm od) coronary arteries of dogs, and confirmed the in situ work suggesting differences in the responses of the vascular smooth muscle of the large and small coronary arteries to GTN. They also found that adenosine causes greater relaxation of the small vessels than the large vessels (17), which is in agreement with our in vitro data for adenosine. Thus, from the present in vitro findings, FK409 is expected to facilitate redistribution of the blood to the ischemic region of the myocardium due to relaxation of the conductive large coronary vessels or collateral vessels.

In fact, FK409 produced a favorable redistribution of myocardial blood flow in the present in vivo study (Table 2). In this study, an i.v.-infusion dose (0.32 μg/kg/min) was used to obtain a stable plasma concentration of around 3 ng/ml, which was the effective level in the present ischemic heart models after i.v. bolus injection of FK409 at 10 μg/kg. This infusion dose produced no significant effects on BP, HR or LV max dp/dt. Increased myocardial blood flow in the ischemic region theoretically depends on the drug action on large conductance coronary arteries and collateral vessels rather than on direct effects at the arteriolar level (22). In this case, FK409 was administered after a 30-min stabilization period. As it has been reported that most of the flow increase due to opening of endogenous collateral vessels occurred during the first 5 to 10 min after coronary occlusion in dogs and that ischemic flow was stable during the next several hours (24), it is probable that the compound effects in the present study were upon a stable endogenous collateral system.

Thus, one possible explanation for the anti-ischemic heart effect of FK409 might be its distinctive coronary vasodilating activity. However, it has been reported that FK409 decreased venous return in anesthetized dogs when measured as a sum of the flow through the inferior and the superior vena cava (25), suggesting that the compound also may improve myocardial ischemia through a reduction of cardiac preload.

Yamada et al. (2) reported that the concentration-vasorelaxation curve for FK409 was shifted to the right by methylene blue, an inhibitor of soluble guanylate cyclase, and to the left by M&B 22,948, an inhibitor of cyclic GMP phosphodiesterase. FK409 also produced an increase in cyclic GMP levels associated with a vasorelaxation in isolated dog coronary arteries (2) and isolated rat aortas (8). In addition, it has been reported that the compound released nitric oxide and activated soluble guanylate cyclase even in the absence of SH groups (8). Therefore, it is highly likely that the vasorelaxant effect of FK409 is due to cyclic GMP accumulation. The large coronary selectivity of FK409 may be derived from the
regional difference in cyclic GMP accumulation, although further studies are needed to clarify the detailed mechanisms of vascular selectivity of the compound.

In summary, our in vivo and in vitro studies demonstrated that FK409 suppresses ischemic ECG changes in anesthetized animals, and this suppressive effect is likely to be due to the compound's strong large coronary artery-selective vasorelaxant action. However, other pharmacological actions, such as reduction of cardiac preload, would be possible as additional mechanisms for the anti-ischemic heart effect of the compound.

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