Effect of KRN2391, a Novel Vasodilator, on Various Experimental Anginal Models in Rats

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ABSTRACT—The antianginal effect of KRN2391, N-cyano-N′-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethanesulfonate, on various anginal models in rats was compared with those of nifedipine and nicorandil. Angina pectoris was induced by methacholine or isoproterenol, and the change in the ST-segments in the electrocardiogram (ECG) was used as the parameter to indicate angina pectoris. The intracoronary administration of methacholine (3 μg) produced an elevation in the ST-segment of the ECG. This ST-elevation was inhibited by the intravenous administration of KRN2391 (30 and 100 μg/kg), nifedipine (100 and 300 μg/kg) and nicorandil (1000 and 3000 μg/kg). The administration of isoproterenol (10 μg/kg/min, i.v.) produced a depression of the ST-segment of the ECG. The intravenous administration of KRN2391 (100 μg/kg), nifedipine (100 μg/kg) and nicorandil (3000 μg/kg) inhibited the ECG changes induced by isoproterenol. These results suggest that KRN2391 exerts a potent protective effect on angina pectoris models compared with nifedipine and nicorandil. KRN2391 appears to be useful as an antianginal drug.

Keywords: KRN2391, Coronary vasodilator, Angina pectoris, K channel opener, ECG-change

KRN2391, N-cyano-N′-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethanesulfonate, is a newly synthesized agent that produces potent coronary vasodilation without affecting myocardial functions except at high doses (1). In the in vivo experiment using anesthetized dogs, KRN2391 produced a preferential increase in coronary blood flow (2) following a decrease in myocardial oxygen consumption and an increase in the oxygen supply to the heart (3). These profiles of KRN2391 are thought to be favorable for the therapy of ischemic heart diseases. Presently, nitrates, β-adrenoceptor blockers, calcium channel blockers and nicorandil possessing both a nitrate action and a K channel opening action have been used for the clinical treatment of ischemic heart diseases such as angina pectoris (4–9). In particular, the antianginal effects of nitrates, Ca channel blockers and nicorandil are partly based on their coronary vasodilating actions. The coronary vasodilating mechanism of KRN2391 has been shown to be due to its dual mechanism of action as a nitrate and K channel opener hybrid (N-K hybrid) (10).

In the present study, we examined whether KRN2391 is actually effective on two experimental models of angina pectoris to investigate the possible use of KRN2391 as an antianginal drug. In addition, the effect of KRN2391 was compared with those of nifedipine, a Ca channel blocker (11), and nicorandil which has a dual mechanism of action similar to that of KRN2391 (12).

MATERIALS AND METHODS

Experimental animals

Male Wistar strain rats (weighing 230–330 g) were used in the methacholine- and isoproterenol-induced angina models. The animals were anesthetized with urethane and α-chloralose (1 g/kg, 25 mg/kg, i.p., respectively). The change of the ST-segments of the lead II electrocardiogram (ECG) was used as the parameter indicating ischemic changes in the heart. The amplitude of the ST-changes was determined by the method of Hiramatsu et al. (13).

Effects on methacholine-induced angina model in rats

Experiments were performed according to the method of Sakai et al. (14), with modifications. To inject methacholine at the ostia of the left and right coronary arteries (i.c. administration), a cannula was inserted to the aortic valve through the right carotid artery. This cannula was connected to a microsyringe for i.c. administration. Methacholine was injected over a 1-sec period at a volume...
of 10 µl. First, the change in ECG induced by methacholine was observed as a control observation. Secondly, 20–30 min after the control observation, vehicles or test drugs were intravenously administered by bolus injection 2 min before the injection of methacholine, and the effects of the test drugs on the methacholine-induced change in ECG were observed. The lead II ECG was continuously recorded with an electrocardiograph (AP-621G; Nihon Kohden, Tokyo).

**Effects on isoproterenol-induced angina model in rats**

Isoproterenol (10 µg/kg/min) was infused intravenously through the cannula inserted into the right or left femoral vein. Test drugs or their vehicles were intravenously administered by bolus injection 2 min before the administration of isoproterenol. In this series, the effects of vehicles and test drugs were examined in different animals. Lead II ECG was recorded for 5 min after infusion by an electrocardiograph (AB-621G, Nihon Kohden).

**Drugs**

KRN2391 and nicorandil were synthesized in our laboratory. Methacholine chloride, (-)-isoproterenol hydrochloride and nifedipine were purchased from Sigma (St. Louis, MO, USA). Methacholine chloride, (-)-isoproterenol, KRN2391 and nicorandil were dissolved in saline. Nifedipine was dissolved in 0.9% saline/polyethylene glycol 400/ethanol (70:15:15, vol./vol.) and then diluted with the same medium.

**Statistical analyses**

Values are shown as the mean±S.E.M. Differences were considered significant when P<0.05 by the paired Student’s t-test or analysis of variance for multiple comparisons. When multiple comparisons were made using a single control, Dunnett's test was used to determine the level of significance.

**RESULTS**

**Effects on methacholine-induced angina model in rats**

When methacholine (3 µg) was administered at the ostia of coronary arteries in rats, an elevation of the ST-segment was observed on lead II ECG in all rats. The ECG changes lasted for 5–10 sec. A typical tracing of the effect of KRN2391 on methacholine-induced ST-elevation is shown in Fig. 1. The intravenous administration of KRN2391 (30 and 100 µg/kg), nifedipine (100 and 300 µg/kg) and nicorandil (1000 and 3000 µg/kg) significantly inhibited the methacholine-induced ST-elevation (Fig. 2). The inhibitory effect of KRN2391 was at a lower dose in comparison with those of nifedipine and nicorandil.
Effects of i.v. administered KRN2391, nifedipine and nicorandil on methacholine-induced ST-elevation in rats. Each value shows the peak change in the ST-segment induced by methacholine. Each column represents the mean±S.E.M. of five rats that were not treated (□) or treated with the drug (■). *P<0.05: Significantly different from the values before drug administration.

Effects on isoproterenol-induced angina model in rats

When isoproterenol (10 μg/kg/min) was intravenously infused, a depression of the ST-segment in the lead II ECG appeared 30 sec after initiation of the infusion. The maximal ST-depression was 0.26±0.03 mV in rats given saline and 0.29±0.04 mV in rats given the vehicle for nifedipine. A typical tracing of the effect of KRN2391 on isoproterenol-induced ST-depression is shown in Fig. 3. The intravenous administration of KRN2391 (100 μg/kg) significantly inhibited the isoproterenol-induced ST-depression (Fig. 4A). Nifedipine (100 μg/kg) and nicorandil (3000 μg/kg) also showed significant protective effects against isoproterenol-induced ST-depression (Fig. 4, B and C). In these doses, the inhibitory effects of KRN2391 and nicorandil were larger than that of nifedipine.

Fig. 3. Typical tracings of changes in the electrocardiogram (lead II) induced by isoproterenol (10 μg/kg/min, i.v.) in rats and the effects of KRN2391.
DISCUSSION

It is well known that angina pectoris is a clinical syndrome caused by myocardial ischemia resulting from an imbalance between myocardial oxygen supply and demand. This imbalance is thought to be due to a decrease in oxygen supply to the heart caused by a decrease in coronary blood flow and an increase in oxygen demand caused by positive chronotropic and/or inotropic actions in addition to constriction of the coronary arteries (15, 16). Myocardial ischemia is closely reflected by the changes of the ST segment in the ECG, and the ECG is an important diagnostic method for anginal pectoris (17). As experimental animal models reflecting the clinical picture, angina models induced by drugs such as methacholine and isoproterenol are used mostly on the basis of electrocardiographic studies. From the mechanism of action of methacholine and isoproterenol and the changes of the ST-segment in induced by these drugs, methacholine and isoproterenol are thought to reflect clinical variants of angina pectoris and angina of effort, respectively. The present study showed that KRN2391 as well as nifedipine and nicorandil, which are clinically used as antianginal drugs, was effective on anginal models induced by methacholine and isoproterenol, suggesting that KRN2391 may be useful in the therapy of angina pectoris.

It is reported that the intracoronary administration of methacholine produces coronary vasoconstriction and induces ST-elevation of the electrocardiogram associated with an ischemia to the heart (18, 19). Therefore, methacholine-induced anginal models are thought to reflect the ST-elevation in patients with variant angina based on coronary vasospasm. The intravenous administration of KRN2391 (3), nicorandil (20) and nifedipine (21) have been observed to increase the oxygen supply to the heart via an increase in coronary blood flow and a decrease in total vascular resistance in anesthetized dogs. In isolated vascular smooth muscle, these drugs also showed a direct relaxant effect on contraction caused by various vasoconstrictors (9, 10, 22-24). Therefore, the antianginal effects of KRN2391, nifedipine and nicorandil are thought to be due to their inhibitory action on the coronary artery contraction induced by methacholine.

Isoproterenol, a β-adrenoceptor agonist, is known to augment myocardial oxygen consumption (25). This is based on the positive chronotropic and inotropic effects of isoproterenol. In addition, it is reported that intravenous infusion of isoproterenol induced a depression of the ST-segments of the ECG as a parameter for indicating an anginal attack according to the increase in myocardial oxygen consumption in rats (26). The isoproterenol-induced anginal model in the present study seems to reflect the clinical findings. Although KRN2391, nifedipine and nicorandil increased the oxygen supply to the heart as mentioned above, they showed different effects on myocardial oxygen consumption. In anesthetized dogs, myocardial oxygen consumption was decreased by KRN2391 (3) and not affected by nicorandil (20). However, different results have been reported for nifedipine in which it produced a decrease (21), an increase (27) or no change (3, 28) in the myocardial oxygen consumption of dogs. Therefore, the effects of these drugs on myocardial oxygen consumption may not contribute to their protective effects on the isoproterenol-induced model in rats. The protective effects of these drugs on the isoproterenol-induced model as well as methacholine-induced
model appear to be due to the increase in oxygen supply via an increase in coronary blood flow and a reduction in afterload on the heart via a decrease in total vascular resistance.

In conclusion, KRN2391 is expected to be useful in the therapy of angina pectoris because KRN2391 as well as nifedipine and nicorandil showed protective effects in various angina models.

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