J. Pharm. Pharmacol. 1994, 46: 558–562
Received September 6, 1993
Accepted January 18, 1994

U-46619-induced Ischaemic Electrocardiographic Changes in Rats: Preventive Effects of Prostacyclin and Nitroglycerin

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Abstract—The anti-anginal effect of nitroglycerin and prostacyclin was examined using, as an index, the ischaemic electrocardiogram (ECG) change (ST elevation) induced by intracoronary arterial injection of 9,11-dideoxy-11α,9α-epoxymethano-$PGF_{2\alpha}$ (U-46619), a stable thromboxane A_2 agonist, in anaesthetized rats. The ST elevation induced by U-46619 (5–20 $\mu g\,kg^{-1}$, i.c.a.) was dose-dependent and reproducible. U-46619-induced ST elevation was markedly prevented by the pretreatment of intravenous administration of prostacyclin (0·01 $\mu g\,kg^{-1}$), and to a lesser extent by nitroglycerin (0·3 $mg\,kg^{-1}$). Simultaneously, platelet count decreased significantly in the coronary arterial blood which indicated that platelet aggregation was enhanced by U-46619. The decrease of platelet count in coronary arterial blood at the time of ST elevation was significantly suppressed by prostacyclin (0·1 $\mu g\,kg^{-1}$, i.v.), but not by nitroglycerin (0·3 $mg\,kg^{-1}$, i.v.). These results suggest that the ST elevation induced by intracoronary arterial injection of U-46619 may be derived from spasm of coronary artery and platelet aggregation in the intracoronary artery in rats.

Vasoactive substances, such as vasopressin, methacholine, 5-hydroxytryptamine, histamine and ergonovine, constrict coronary artery in various experimental animals (Hiramatsu et al 1970; Sakai et al 1981; Shimokawa et al 1983; Kawachi et al 1984). These substances, however, were found to be inadequate as inducers of experimental angina. For instance, the myocardial ischaemic change caused by vasopressin is gradually decreased by repeated administration (Hiramatsu et al 1970; Lolait et al 1992; Morel et al 1992; Ostrowski et al 1992), and methacholine brings out a marked systemic hypotension at the time of ischaemic ECG change (ST elevation) after intracoronary arterial (i.c.a.) injection. 5-Hydroxytryptamine, histamine and ergonovine could produce spasms in rodents, because of marked species difference in development of vasospasms (Shimokawa et al 1983; Kawachi et al 1984). Accordingly, the present study sought a more reliable inducer of angina pectoris by examining the influence on electrocardiogram (ECG) in anaesthetized rats.

Recently, it was noted that prostanoids regulate both the tonus of vasculature and the function of platelets, and that an imbalance of endogenous contents of thromboxane A_2 (TXA_2) and prostacyclin can bring about spasm and thrombus in the coronary artery, decreasing oxygen supply to the myocardium (Moncada & Vane 1979; Tada et al 1984; Yui et al 1987). It was also reported that TXA_2 is involved in the development of angina pectoris in man (Halushka & Mais 1989). However, TXA_2 is not suitable as an inducer in experimental animals because of its lability (Tada et al 1984).

A stable TXA_2 agonist, U-46619, was found to possess potent vasoconstrictive activity (Coleman et al 1981; Schumacher & Heran 1989; Templeton et al 1991). It was reported that this substance decreases coronary blood flow after intracoronary arterial administration (Kopia et al 1987; Hom et al 1992). U-46619 also has potent platelet aggregative activity (Halushka & Mais 1989) and then produces thrombus after intracoronary arterial administration (Schumacher et al 1987), whereas methacholine, a traditional inducer, had no platelet aggregative activity at 100 times the concentration in rat plasma that brought about ST elevation (unpublished data). Therefore, it seems likely that a new experimental model of angina pectoris where both constriction of the coronary artery and intracoronary aggregation of platelets take place can be prepared using U-46619. Such a model would be mechanistically much closer to angina pectoris in man than the previously reported traditional models which derive from the constriction of the coronary artery only (Hiramatsu et al 1970; Sakai et al 1981).

In the present study, we examined the conditions under which U-46619 induced the ST elevation after i.c.a. administration in rats, and we tested prostacyclin and nitroglycerin for their efficacy against the ischaemic change to assess the significance of the pathological state in this new experimental model.

Materials and Methods

Induction of ischaemic electrocardiogram change

Male Sprague Dawley rats, 400–500 g, were anaesthetized with sodium thiamylal (50 $mg\,kg^{-1}$, i.p.). To facilitate spontaneous respiration, a cannula (polyethylene tube No. 7, Hibiki, Tokyo, Japan) was inserted into the trachea. A cannula (polyethylene tube PE-50, Becton Dickinson, New Jersey, USA) for injecting the inducer was inserted through the right common carotid artery and the tip of the cannula was positioned at the ostia of the coronary artery. This cannula was fixed at the position where the ST elevation of 0·2 mV or more was confirmed after administration of methacholine (16 $\mu g\,kg^{-1}$, i.c.a.). ECG (lead II) was recorded by an electrocardiograph (ECG-6601, Nihon

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Kohden, Tokyo, Japan). After 15 to 30 min, all parameters were stable, and the experiment was started. ECG was recorded for 10 s at 30-s intervals for 5 min after administration of U-46619 and followed, thereafter, at 5-min intervals. Induction of the ischaemic ECG change caused by methacholine was performed essentially according to the method of Sakai et al (1981) and Yamamoto et al (1993). The injected amount of methacholine into the coronary artery was set as 16 $\mu g\,kg^{-1}$.

Test drugs were injected intravenously into the femoral vein 1 and 0·5 min before injection of U-46619 (10 $\mu g\,kg^{-1}$, i.c.a.) and methacholine (16 $\mu g\,kg^{-1}$, i.c.a.), respectively.

Platelet aggregation

Platelet aggregation was evaluated by measurement of platelet count in the coronary arterial blood. According to the method of Deguchi et al (1972), 50 μL blood was collected in the presence of 3·8% citric acid through the cannula positioned at the ostia of the coronary artery, before and at 1·5 and 21·5 min after administration of U-46619 and then 2 μL collected blood was diluted with 10 mL Cellent (CE-310, Toa-Iyou-Denshi, Tokyo, Japan). The diluted solution was centrifuged at 900 rev min^{-1} for 20 min and measured by an automatic platelet counter (PL-100, Toa-Iyou-Denshi). The preventive effect of prostacyclin and nitroglycerin on platelet aggregation was determined as follows. After the ECG change from the first challenge of U-46619 (10 $\mu g\,kg^{-1}$, i.c.a.) or methacholine (16 $\mu g\,kg^{-1}$, i.c.a.) had recovered completely, the test drug was injected into the femoral vein, and the second challenge of U-46619 or methacholine was given at 1 or 0·5 min after the administration of the test drug, respectively.

Measurements of blood pressure and heart rate

A polyethylene tube (PE-50) was inserted into the left femoral artery in anaesthetized rats, and mean blood pressure and heart rate were measured by a pressure transducer (TNF-R, Nihon Kohden) and a polygraph (AP-641G, AP-601G, Nihon Kohden).

Drugs

U-46619 (9,11-dideoxy-11α,9α-epoxymethano-$PGF_{2\alpha}$, Funakoshi, Tokyo, Japan) was dissolved in ethanol and diluted with 0·9% NaCl. Prostacyclin (Sigma, St Louis, MO, USA) was dissolved in ethanol and diluted with 0·1 M glycine-NaOH buffer (pH 10·0). Methacholine (Sigma, St Louis, MO, USA) and nitroglycerin (Green Cross, Osaka, Japan) were dissolved in 0·9% NaCl.

Statistical analysis

The results are expressed as means ± s.e. The statistical significance of differences were evaluated by Student's *t*-test. The statistical significance of reproducibility in the value of ST elevation for the first and second challenges was also assessed using a one-way analysis of variance and the least significant difference method. $P < 0.05$ was considered to be significant.

Results

Induction of ischaemic electrocardiogram change

Intracoronary arterial administration of U-46619 at the dose of 2·5 $\mu g\,kg^{-1}$ showed no change in ECG as did the saline control. At doses larger than 5 $\mu g\,kg^{-1}$, U-46619 caused an elevation of ST segment in a dose-dependent manner (Figs 1, 2). At a dose of 10 $\mu g\,kg^{-1}$, the maximum change of ST segment reached 0·20 ● 0·04 mV at 1·5 min after U-46619 injection. The elevation became significant as early as 30 s after the injection and lasted for about 2·5 min,

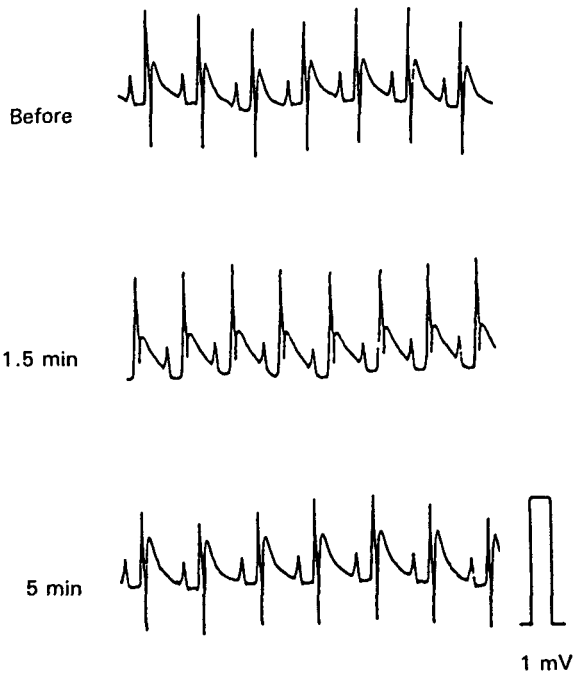

FIG. 1. Typical traces showing the change in ST segment before and at 1·5 and 5 min after administration of U-46619 (10 $\mu g\,kg^{-1}$, i.c.a.) in an anaesthetized rat.

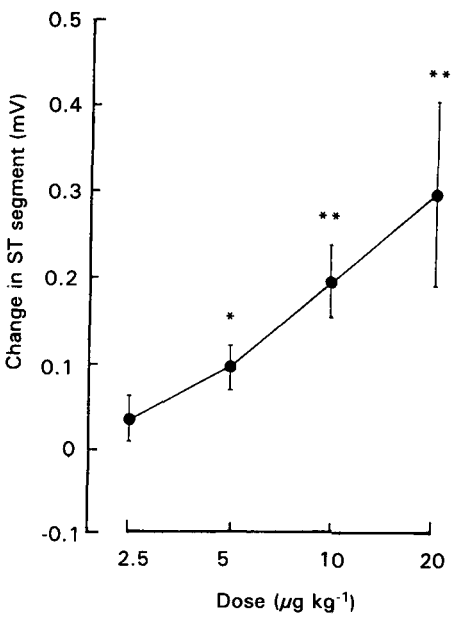

FIG. 2. Dose-dependency of ST elevation induced by U-46619 (2·5–20 $\mu g\,kg^{-1}$, i.c.a.) in anaesthetized rats (n = 7–13). Each point and vertical line represents the mean ± s.e. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the values before treatment.

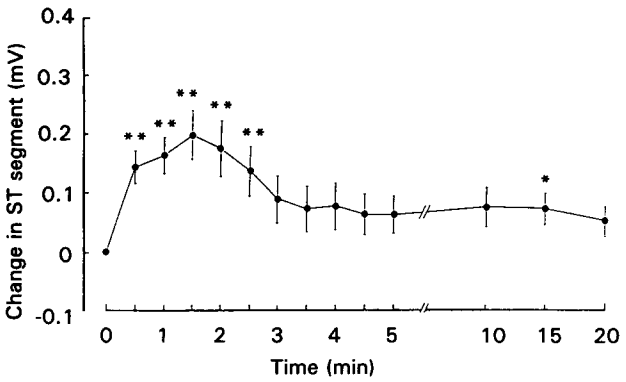

FIG. 3. Time-dependency of ST elevation induced by U-46619 (10 $\mu g\,kg^{-1}$, i.c.a.) in anaesthetized rats (n = 13). Each point and vertical line represents the mean ± s.e. $^*P < 0.05$, $^{**}P < 0.01$ compared with the values before treatment.

followed by a rapid recovery (Fig. 3). The changes of the ST segment from 0·5 to 5 min after the second challenge of U-46619 did not significantly differ from those of the first challenge. Based on these results, the dose of 10 $\mu g\,kg^{-1}$ U-46619 was assumed to be suitable for the following experiments. Methacholine, when given intracoronarily at a dose of 16 $\mu g\,kg^{-1}$, caused an immediate elevation of ST segment, as reported by Yamamoto et al (1993).

Preventative effect of prostacyclin and nitroglycerin on ischaemic electrocardiogram change

Intravenous administration of prostacyclin significantly prevented the U-46619- and methacholine-induced ST elevation at doses of 0·01 and 0·1 $\mu g\,kg^{-1}$, respectively (Table 1). Nitroglycerin also significantly prevented the U-46619- and methacholine-induced ST elevation at intravenous doses of 0·3 and 0·1 $mg\,kg^{-1}$, respectively (Table 1).

Effect of U-46619, prostacylin and nitroglycerin on platelet aggregation

In the control period, the number of platelets was $630·3 \pm 64·8 \times 10^3 mm^{-3}$; 1 min after administration of U-46619, this decreased to $433·0 \pm 47·3 \times 10^3\ mm^{-3}$, indicating that U-46619 elicited aggregation of platelets in the intracoronary artery. The decrease of platelet count was unaffected by a second administration of U-46619. Intravenous prostacyclin significantly inhibited the decrease of platelet count at a dose of 0·1 $\mu g\,kg^{-1}$ (Table 2), whereas intravenous nitroglycerin was without effect even at a dose of 0·3 $mg\,kg^{-1}$ (Table 2).

Effects of U-46619 and methacholine on blood pressure and heart rate

The typical blood pressure changes caused by U-46619 and methacholine are shown in Figs 4 and 5. U-46619 (10 $\mu g\,kg^{-1}$, i.c.a.) increased the blood pressure and maintained it at high level for 5 min. Methacholine (16 $\mu g\,kg^{-1}$, i.c.a.) caused prompt hypotension with a return to control levels within 3 min. The time course of mean blood pressure after administration of U-46619 or methacholine is shown in Fig. 6.

U-46619 increased heart rate by about 25 beats min^{-1}, and methacholine transiently decreased heart rate by about 200 beats min^{-1}.

Discussion

In the present study, it was found that U-46619 produced the ischaemic ECG change (ST elevation) by intracoronary arterial administration in rats. This ST elevation is similar to the ischaemic ECG change in variant angina pectoris, suggesting that U-46619 induces epicardial ischaemia by coronary arterial spasms.

This ST elevation, like the methacholine-induced ST elevation, was effectively inhibited by the traditional anti-anginal drug nitroglycerin which produces an anti-anginal action by dilating the coronary arteries. Therefore, the vasoconstrictive activity of U-46619 may be a mechanism for its induction of cardiac ischaemia. This suggestion is supported by Yui et al (1987) who showed that thiathromboxane A_2 (STA_2), a TXA_2 agonist, constricted the coronary artery and decreased coronary blood flow after coronary arterial administration in rabbits.

The ST elevation induced by U-46619 was more effectively prevented by prostacyclin, which has anti-platelet-aggregative activity and vasodilative activity at the lower dose. This result suggests that platelet function is enhanced by U-46619, which in turn may be involved in the ST elevation as a modifying factor. Collagen (1 $mg\,kg^{-1}$, i.v.), which has potent platelet aggregative activity but does not have vasco-

Table 1. Preventive effects of prostacyclin and nitroglycerin on U-46619- or methacholine-induced ST elevation in anaesthetized rats.

Test drugs	Dose ($\mu g\,kg^{-1}$, i.v.)	Δ ST (mV)			
		U-46619 (10 $\mu g\,kg^{-1}$, i.c.a.)		Methacholine (16 $\mu g\,kg^{-1}$, i.c.a.)	
		Control	Test drug	Control	Test drug
Prostacyclin	0·1	0·24 ± 0·04	0·10 ± 0·04*	0·23 ± 0·04	0·10 ± 0·02*
	0·01	0·25 ± 0·04	0·12 ± 0·03*	0·29 ± 0·03	0·22 ± 0·02
	0·001	0·20 ± 0·05	0·19 ± 0·02		
Nitroglycerin	300	0·13 ± 0·02	0·06 ± 0·01**		
	100	0·16 ± 0·04	0·12 ± 0·02	0·20 ± 0·02	0·07 ± 0·03*
	30			0·20 ± 0·03	0·13 ± 0·04

Drugs were administered according to the methods in the text. Results are expressed as means ± s.e. obtained from 4 to 5 rats. Statistical significance of a difference in the value of ST segment between control and test drug was evaluated by means of Student's *t*-test. $^*P < 0.05$, $^{**}P < 0.01$ vs control.

Table 2. Preventive effect of prostacyclin and nitroglycerin on U-46619-induced platelet aggregation in anaesthetized rats.

Test drugs ($\mu g\,kg^{-1}$, i.v.)	Platelet count ($\times 10^3\,mm^{-3}$)	Percent change
Before	767·0 ± 51·2	
Solvent + U-46619	518·5 ± 21·9**	−32·4
Prostacyclin (0·1) + U-46619	669·3 ± 11·6†	−12·7
Before	669·4 ± 25·4	
Solvent + U-46619	485·6 ± 43·6**	−27·5
Nitroglycerin (300) + U-46619	484·2 ± 30·1**	−27·8

Solvent, prostacyclin and nitroglycerin were intravenously administered 1 min before U-46619 ($10\,\mu g\,kg^{-1}$, i.c.a.) in anaesthetized rats (n = 5). Results are expressed as means ± s.e. Statistical significance of a difference in the value of platelet count between before and after administration of solvent or test drugs was evaluated by analysis of variance and least-significant difference methods. $^{**}P < 0.01$ compared with before value, $^{\dagger}P < 0.05$ compared with solvent value.

constrictive activity, is known to produce an elevation of ST segment in rats (Matsumura et al 1986). Therefore, we examined whether U-46619 induced platelet aggregation in the coronary artery and, if so, whether nitroglycerin as well as prostacyclin could prevent the aggregation. The platelet count after intracoronary arterial injection of U-46619 was significantly decreased when compared with the count before U-46619 injection. This decrease in platelet count was significantly prevented by pretreatment with intravenous prostacyclin at $0.1\,\mu g\,kg^{-1}$, the dose which was effective in suppressing the ST elevation. On the other hand, the decrease in platelet count could not be prevented by intravenous nitroglycerin at $0.3\,mg\,kg^{-1}$, the effective dose against the ST elevation. Taking these results and those of Matsumura et al (1986) mentioned above, it can be assumed that the enhancement of platelet function is involved in the mechanisms by which U-46619 causes the elevation of ST segment. The fact that U-46619 has potent platelet-aggregative activity is compatible with this assumption.

There are two types of drug-induced angina model. One comprises those induced by vasopressin or methacholine, where the change in cardiac function is due to the decreases in coronary blood flow and oxygen supply to the myocardium (Hiramatsu et al 1970; Sakai et al 1981; Yamamoto et al 1993), and the other comprises those induced by isoprenaline where the mechanism involves increases in heart rate and myocardial oxygen demand (Karasawa et al 1988). Although these two types of angina models have been used for evaluating anti-angina candidate compounds, their usefulness has not been fully assured because of occurrence of some crucial extracoronary arterial changes such as severe hypotension.

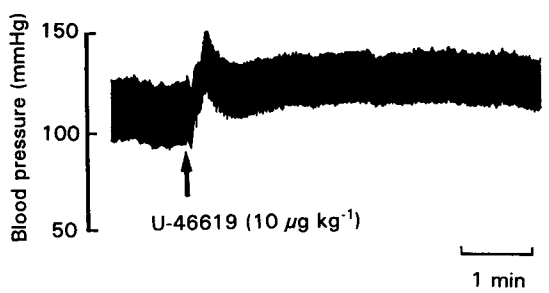

FIG. 4. Typical traces showing the change in blood pressure induced by U-46619 ($10\,\mu g\,kg^{-1}$, i.c.a) in an anaesthetized rat.

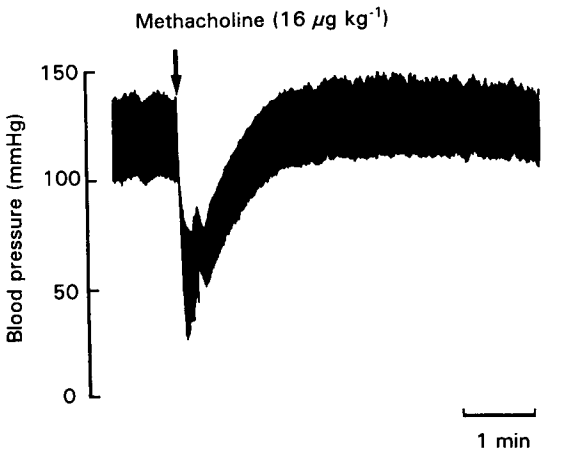

FIG. 5. Typical traces showing the change in blood pressure induced by methacholine ($16\,\mu g\,kg^{-1}$, i.c.a.) in an anaesthetized rat.

For example, methacholine ($16\,\mu g\,kg^{-1}$, i.c.a.) which was widely used as an ST elevation inducer produced a marked fall of about 60 mmHg in blood pressure and a remarkable decrease of about 200 beats min^{-1} in heart rate. In this

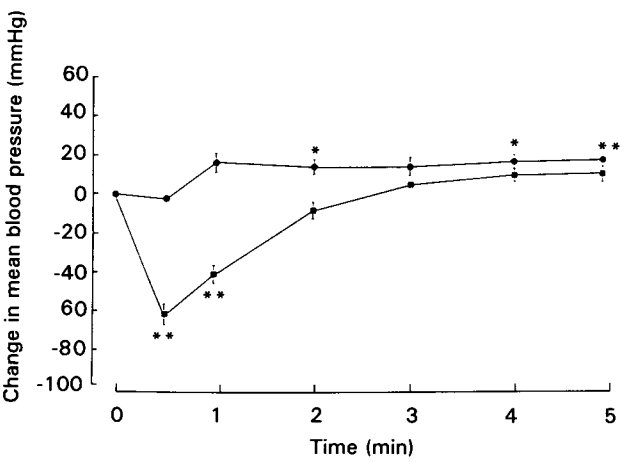

FIG. 6. Effect of U-46619 and methacholine on mean blood pressure in anaesthetized rats (n = 5). ● U-46619 ($10\,\mu g\,kg^{-1}$, i.c.a.), ■ methacholine ($16\,\mu g\,kg^{-1}$, i.c.a.). Each point and vertical line represents the mean ± s.e. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with values before treatment.

respect, U-46619 ($10\,\mu g\,kg^{-1}$) had little effect, the increases in blood pressure and heart rate being only about 15 mmHg and 25 beats min^{-1}, respectively, with no other circulatory changes being observed. Thus, it is possible that U-46619 induces the ischaemic change in cardiac tissue without crucial haemodynamic effects.

In conclusion, the ST elevation induced by U-46619 provides a novel experimental angina model for evaluating new anti-anginal drugs.

Acknowledgements

The authors are particularly grateful to Dr. T. Karasawa and Dr Y. Masuda for their valuable advice and encouragement throughout the work.

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