Inhibitory Effect of a Novel Antianginal Agent, E4080, on ST Segment Elevation Induced by Vasopressin in Anesthetized Guinea Pigs

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ABSTRACT — We compared the antianginal effect of E4080, a novel bradycardic agent with coronary vasodilating properties, with those of a bradycardic agent and some coronary vasodilators in vasopressin-induced anginal model of guinea pigs. An i.v.-administration of vasopressin (0.2 IU/kg) produced an ST segment elevation on electrocardiograms (ECG) of 0.30 ± 0.05 mV from the baseline within 30 sec in anesthetized guinea pigs. The ST segment elevation on ECG was used as an index of myocardial ischemia. E4080 and other drugs were injected i.v. 5 min before the administration of vasopressin. E4080 at 5 mg/kg depressed the ST segment elevation induced by vasopressin to 0.06 ± 0.01 mV (20% of control, n = 6, P < 0.001). However, alinidine (5 mg/kg), which produced the same bradycardic action (reduction of heart rate by 50%) as that of E4080, tended to inhibit the ST segment elevation, but this was not statistically significant. On the other hand, other vasodilators such as isosorbide dinitrate (0.3 mg/kg), nifedipine (0.1 mg/kg) and lemakalim (1 mg/kg) also significantly reduced the ST segment elevation to 0.16 ± 0.03, 0.08 ± 0.04 and 0.09 ± 0.03 mV, respectively. These results suggest that the inhibitory effect of E4080 on the ST segment elevation induced by vasopressin is due to the coronary vasodilating effect rather than the bradycardic effect, and that E4080 would be useful as an antianginal agent.

Keywords: E4080, Bradycardic agent, Coronary vasodilator, Vasopressin, ST segment elevation

Angina pectoris is evoked by an imbalance between the supply and demand for oxygen of the heart. Clinically, the electrocardiogram (ECG) is an important diagnostic method for ischemic heart disease, because myocardial ischemia is closely reflected by changes on ECGs such as elevation or depression in the ST segment (1).

Vasopressin, an endogenous vasoconstrictor and antidiuretic hormone, produces coronary vasoconstriction, which leads to myocardial ischemia, as characterized by depressed myocardial function (2–5). Takeda et al. (6) have reported that in anesthetized guinea pigs, i.v.-administration of vasopressin produces a prominent elevation in the ST segment and that nitroglycerin significantly inhibited the ECG change, indicating that this animal model is useful for evaluation of antianginal drugs.

E4080, (E)-N-[3-(((N’-(2-(3,5-dimethoxyphenyl)ethyl)-N’-methyl)amino)propyl]-4-(4-(1H-imidazol-1-yl)-phenyl)-3-butenamid·2HCl·2H2O, is a novel bradycardic agent with coronary vasodilating properties (7, 8), which was developed as an antianginal drug. E4080 produces both bradycardic and hypotensive actions that lead to a decrease in myocardial oxygen consumption in anesthetized dogs (9). In addition, E4080 directly dilates not only the small coronary artery but also the epicardial large coronary artery in conscious dogs (10). However, it remained to be determined whether this compound produces an antianginal effect in an experimental anginal model.

In the present study, we examined the inhibitory effect of E4080 on vasopressin-induced ST segment elevation and compared it with the effects of a bradycardic agent and some coronary vasodilators in anesthetized guinea pigs. We used alinidine as a bradycardic agent and isosorbide dinitrate (ISDN), nifedipine, and lemakalim (a K channel opener) as coronary vasodilators.
MATERIALS AND METHODS

Animals, instrumentation and experimental protocols

Male Hartley guinea pigs, weighing 200–440 g, were fasted overnight, then anesthetized with urethane (1.5 g/kg, i.p.). The carotid artery and the jugular vein were cannulated with a polyethylene tube (i.d., 0.5 mm; o.d., 0.8 mm) to measure arterial pressure and for i.v.-injection of vasopressin and drugs, respectively. A standard limb lead I ECG was continuously recorded with an electrocardiograph (Cardiofax, Nihon Kohden).

After the arterial pressure was stabilized following the completion of surgery, control values for ECG, heart rate and arterial pressure were obtained and the experiments were started. E4080 (1 and 5 mg/kg), alinidine (1 and 5 mg/kg), ISDN (0.1, 0.3 and 1.0 mg/kg), nifedipine (0.03, 0.1 and 0.3 mg/kg), lemakalim (0.1, 0.3 and 1.0 mg/kg) and 0.9% saline as the control were administered i.v. at a volume of 1 ml/kg body weight, 5 min before vasopressin.

To quantify the effect of drugs on ECG, the height of the T-wave over the TQ base was evaluated (TQ-T, in mV). The heart rate was calculated from the R-R interval. The ECG and the arterial pressure were continuously monitored for 1 min after vasopressin administration. The ST segment elevation and heart rate were measured 30, 45 and 60 sec after vasopressin administration and are shown as the means of 3 consecutive waves. We defined this maximum change in the ST segment elevation that occurred within 30 sec after vasopressin administration as the value obtained at 30 sec.

Drugs

E4080, alinidine, ISDN and lemakalim were synthesized at Eisai Tsukuba Research Laboratories. Nifedipine was purchased from Sigma. Vasopressin (Sigma) was dissolved in 0.9% saline at a concentration of 0.2 IU/ml and stored at −20°C until use. Lemakalim and nifedipine were dissolved in 10% polyethylene glycol 200 and 50% ethanol, respectively, to a concentration of 1 mg/ml. E4080, alinidine and ISDN were dissolved in 0.9% saline to a concentration of 1 mg/ml.

Data analyses

All data are presented as means ± S.E.M. The values of the ST segment elevation by vasopressin after drug administration were compared with the control value after saline using one-way analysis of variance. When a significant difference was detected, Duncan's multiple range test was used to determine the statistical significance of the difference between the control value and the value after the drug was given. P values below 0.05 were considered to be statistically significant.

![Fig. 1. Representative tracings of changes on electrocardiograms (lead I) induced by an i.v.-administration of vasopressin and the effects of E4080 and alinidine on the ST segment elevation in anesthetized guinea pigs. Vasopressin at a dose of 0.2 IU/kg was administered i.v. 5 min after 0.9% saline or drug. The ST segment elevation reached the maximum 30 sec after an i.v.-administration of vasopressin. Although E4080 at 5 mg/kg dramatically suppressed the ST segment elevation induced by an i.v.-administration of vasopressin, alinidine at 5 mg/kg did not.](image-url)
RESULTS

Representative tracings of ST segment elevation on ECG after i.v.-administration of 0.2 IU/kg of vasopressin are summarized in Fig. 1. Vasopressin transiently elevated the ST segment by 0.30 ± 0.05 mV (n = 10) from the baseline of TQ within 30 sec (Fig. 2). Then, the mean arterial pressure increased from 45 ± 2 mmHg of the value before vasopressin to 71 ± 2 mmHg, and the heart rate decreased from 299 ± 15 to 268 ± 13 beats/min. These hemodynamic changes persisted for over 60 sec. However, ST segment elevation decreased gradually after it reached the maximum, and it disappeared within 60 sec after vasopressin administration (Fig. 2). Thus, we used the maximum ST segment elevation that occurred within 30 sec after vasopressin administration as an index of myocardial ischemia.

Although E4080 at a dose of 1 mg/kg did not inhibit ST segment elevation, 5 mg/kg significantly inhibited that induced by vasopressin to 0.06 ± 0.01 mV (20% of control, n = 6, P < 0.001), as shown in Fig. 3. At this time, E4080 decreased both mean arterial pressure by 38% (from 45 ± 3 to 28 ± 2 mmHg) and heart rate by 50% (from 316 ± 14 to 157 ± 9 beats/min). On the other hand, although alinidine at 5 mg/kg, which produces bradycardic effects to the same extent as that of E4080 (from 298 ± 11 to 160 ± 6 beats/min), tended to inhibit the ST segment elevation, the change was not statistically significant (Fig. 3).

In addition, the suppressive actions of coronary vasodilators such as ISDN, nifedipine and lemakalim on the ST segment elevation are shown in Fig. 4. ISDN at 0.1 mg/kg tended to inhibit the ST segment elevation, whereas ISDN at 0.3 and 1.0 mg/kg significantly did so, to 0.16 ± 0.03 mV (n = 6, P < 0.01) and 0.14 ± 0.03 mV (n = 6, P < 0.01), respectively. These doses of ISDN did not significantly change the mean arterial pressure and heart rate. Nifedipine at doses of 0.1 mg/kg, which did not influence either mean arterial pressure or heart rate, caused a significant depression of the ST segment elevation to 0.08 ± 0.04 mV (n = 6, P < 0.01). Lemakalim at a dose of 1.0 mg/kg significantly inhibited the ST segment elevation to 0.09 ± 0.05 mV (n = 6, P < 0.01). Unlike ISDN and nifedipine, lemakalim decreased the mean arterial pressure dose-dependently and at 1.0 mg/kg, reduced it from 46 ± 3 to 23 ± 1 mmHg without any changes in heart rate.

![Fig. 2. Time course of changes in ST segment elevation induced by an i.v.-administration of vasopressin. Data are expressed as means ± S.E.M. (n = 10).](image)

![Fig. 3. Effects of E4080 and alinidine on ST segment elevation induced by an i.v.-administration of vasopressin. E4080 at a dose of 5 mg/kg significantly inhibited the ST segment elevation, whereas alinidine at the same dose did not. Data are expressed as means ± S.E.M. (n = 6–10). ***P < 0.001 vs. control.](image)
DISCUSSION

The ST segment change on the ECG is one of the diagnostic parameters for determining ischemic myocardial tissue injury (1). Maroko et al. (11) and Sayen et al. (12) have shown in experimental coronary occlusion of dogs that a correlation exists between the degree of epicardial ST segment elevation and myocardial tissue injury.

In the present study, we used the ST segment change on ECG induced by vasopressin as an index of myocardial ischemia and evaluated the inhibitory actions of E4080 and other drugs on the ECG changes. Vasopressin is not only an antidiuretic hormone, but also a potent vasoconstrictor. Vasopressin produces constriction of the small coronary artery and increases total coronary resistance in dogs (13). Lamping et al. (14) reported that vasopressin constricted the small coronary artery while it minimally dilated large coronary artery in anesthetized cats. Accordingly, vasopressin is considered to be a vasoconstrictor of the small coronary artery. In our study, the i.v.-administration of vasopressin to guinea pigs caused a transient ST segment elevation together with an increase in arterial blood pressure. In this guinea pig model, the ECG changes after vasopressin administration were considered to demonstrate the presence of myocardial ischemia, which may be produced by coronary vasoconstriction as well as an increase in afterload shown by an elevation in arterial pressure.

E4080 at a dose of 5 mg/kg significantly inhibited the ST segment elevation, whereas alinidine, which has the same extent of bradycardic effect, did not. E4080 increases coronary blood flow and decreases heart rate, aortic pressure and myocardial oxygen consumption in anesthetized open-chest dogs (9). In addition, it dilates the epicardial large coronary artery (10) and suppresses exercise-induced tachycardia in conscious dogs (15). It is considered that the pharmacological mechanism of E4080 is to activate the ATP-sensitive K channel, which contributes to coronary and peripheral vasodilation, and that the direct suppression in the rate of discharge of the sino-atrial node leads to a bradycardic effect (8). In the present study, if the bradycardic action of E4080 relates to the suppression in the ST segment elevation induced by vasopressin, alinidine possessing the same bradycardic action as E4080 would have inhibited the ECG change. However, the suppressive action of alinidine on the ST segment elevation was not significant. These results suggest that the vasodilating effect, rather than the bradycardic effect, contributes to the inhibitory action of E4080 on the ST segment elevation induced by vasopressin.

ISDN has a vasodilator effect upon the large coronary artery, and an attenuating effect on preload by dilating veins (16–18). Nifedipine (19) and lemakalim (20, 21) have a vasodilator effect on large and small coronary arteries and an attenuating effect on afterload by dilating peripheral arteries. In this study, coronary vasodilators such as ISDN, nifedipine and lemakalim significantly inhibited ECG changes induced by vasopressin. Although, ISDN and nifedipine had no effect on mean arterial pressure, lemakalim decreased the mean arterial pressure dose-dependently. On the other hand, coronary vasodilation is the common pharmacological property among ISDN, nifedipine, lemakalim and E4080. These facts suggest that coronary vasodilation has an important role in the mechanism of drug-induced inhibition of the ST segment elevation produced by vasopressin in this guinea pig model. In addition,
lemakalim and E4080 decreased arterial pressure at the
dose that inhibited the ST segment elevation. Accord-
ingly, we can not deny that afterload reduction by de-
creasing arterial pressure may be partly responsible for
the inhibitory effect on the ST segment elevation. The
detailed mechanism of the inhibitory action of E4080
on myocardial ischemia evoked by vasopressin deserves
further investigation.

In conclusion, the major finding in the present study
is that E4080 suppresses the ST segment elevation in-
duced by vasopressin in anesthetized guinea pigs, and
this effect may be based upon its coronary vasodilating
effect rather than its bradycardic effect. These results
suggest that E4080 would be useful in the treatment of
angina pectoris.

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