

## Different Chronotropic and Inotropic Effects of EMD 57033 and EMD 53998, $\text{Ca}^{2+}$ Sensitizers, on Isolated, Blood-Perfused Dog Heart Preparations

Manoj Lakhe, Yasuyuki Furukawa\*, Takanori Yonezawa, Masamichi Hirose, Yoshito Nagashima, Yusuke Miyashita and Shigetoshi Chiba

*Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390–8621, Japan*

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**ABSTRACT**—To investigate whether a  $\text{Ca}^{2+}$  sensitizer increases sinus rate, we studied the effects of racemic thiadiazinone, EMD 53998 (a  $\text{Ca}^{2+}$  sensitizer with phosphodiesterase inhibitory action) and its (+)-enantiomer EMD 57033 (a relatively pure  $\text{Ca}^{2+}$  sensitizer) on isolated, blood-perfused spontaneously beating right atria and paced left ventricles of the dogs. EMD 53998 increased sinus rate dose-dependently, but EMD 57033 did not. Both substances increased atrial and ventricular contractile force. Propranolol did not affect the responses to each substance. These results suggest that the  $\text{Ca}^{2+}$  sensitizing action induced by EMD 57033 does not affect pacemaker currents directly.

**Keywords:** EMD 57033,  $\text{Ca}^{2+}$  sensitizer, Sinus rate

Calcium sensitizers increase myocardial contractility by generating more force per given amount of cytoplasmic free calcium. Such an increase in  $\text{Ca}^{2+}$  responsiveness has been claimed for some inotropic compounds, e.g., sulmazole, pimobendan and isomazole (1). However, this is not their predominant pharmacological mechanism of action because they have a pronounced phosphodiesterase enzyme inhibiting activity. Inhibition of phosphodiesterase causes an increase in tissue cyclic AMP followed by increases in sinus rate as well as myocardial contractility in the heart. Sulmazole and pimobendan increased sinus rate and myocardial contractility in isolated dog heart preparations (2). It has been reported that MCI-154 (6-[4-(4'-pyridyl) aminophenyl]-4,5-dihydro-3 (2*H*)-pyridazinone hydrochloride) is a  $\text{Ca}^{2+}$  sensitizer with a very weak phosphodiesterase inhibition (3). However, MCI-154 caused positive chronotropic and inotropic responses in the isolated, perfused dog atrium (4). Thus, it is still uncertain whether a  $\text{Ca}^{2+}$  sensitizer does not increase sinus rate in the heart.

EMD 53998 is a racemic, equimolar mixture of two optical enantiomers, (+)-enantiomer EMD 57033 and (–)-enantiomer EMD 57439 (5). Recent studies in skinned cardiac fibers (6, 7) and isolated papillary muscles

(8) have shown that the  $\text{Ca}^{2+}$ -sensitizing activity is predominantly attributable to the (+)-enantiomer EMD 57033, while inhibition of phosphodiesterase III is mainly due to the (–)-enantiomer EMD 57439. Thus, if EMD 57033 works as a pure  $\text{Ca}^{2+}$  sensitizer, it might not affect the sinoatrial nodal pacemaker activity. In the present study, therefore, we investigated the effects of EMD 57033 and EMD 53998 on the sinus rate, atrial contractile force and left ventricular force in isolated, blood-perfused right atrial and left ventricular preparations of dogs.

Isolated right atria and left ventricles were perfused with heparinized arterial blood from an anesthetized support dog. The details of the isolated, blood-perfused atrial and ventricular preparations of the dog have been described in previous papers (9, 10). Support dogs weighing 13 to 35 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and ventilated artificially through a cuffed tracheal tube with room air by using a respirator (Harvard Apparatus Co., Inc., South Natick, MA, USA). Sodium heparin (500 USP units/kg, i.v.) was administered to each dog at the beginning of the perfusion of the isolated atrial preparation and 200 USP units/kg were given each hour thereafter.

Isolated right atrial or left ventricular preparations were obtained from other mongrel dogs weighing from 9

\* To whom correspondence should be addressed.

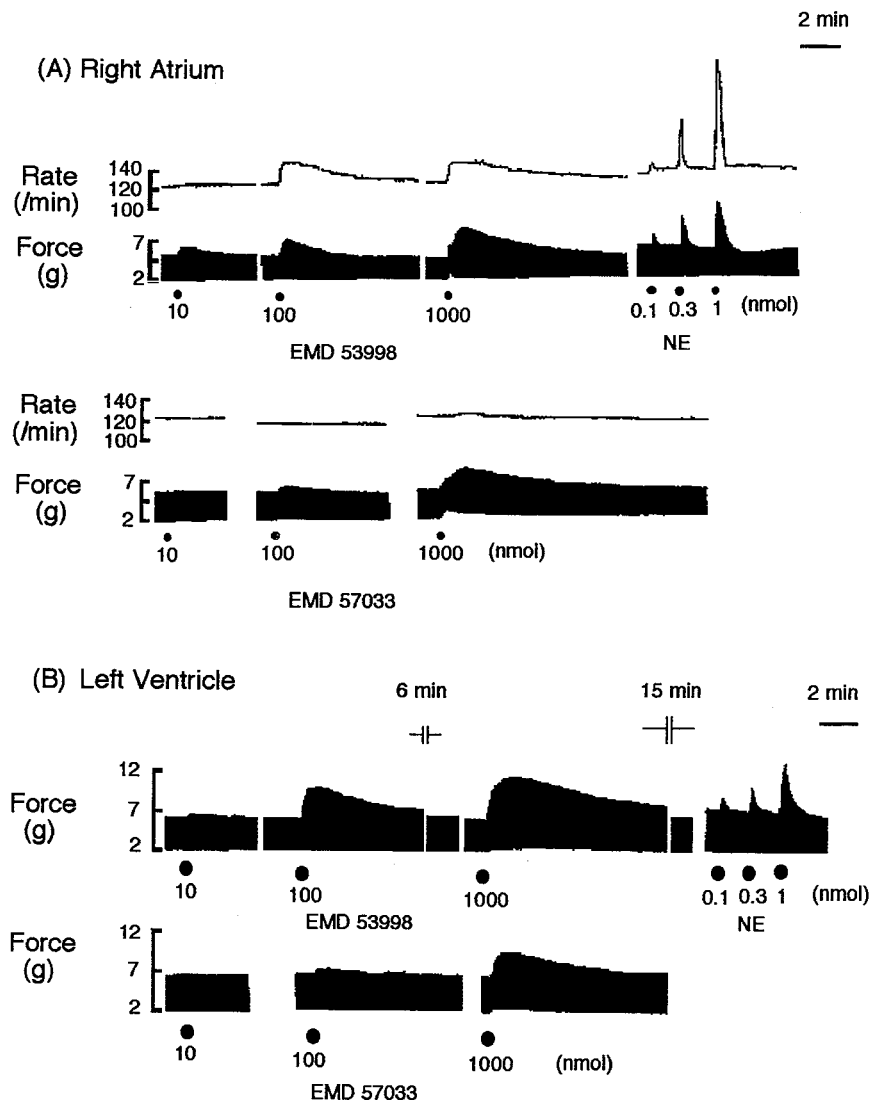


Fig. 1. Effects of EMD 53998, EMD 57033 and norepinephrine on the sinus rate and atrial contractile force in an isolated, blood-perfused canine right atrium (A) and an isolated, blood-perfused canine left ventricle (B). NE, norepinephrine.

to 13 kg. Each dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The right atrium or the left ventricle was excised and immersed in cold Ringer's solution. The sinus node artery of the isolated right atrium or the anterior descending branch of the left coronary artery of the isolated ventricle was cannulated, and each preparation was perfused with heparinized blood from the carotid artery of the anesthetized support dog by the aid of a peristaltic pump (Harvard Apparatus). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained constant at 100 mmHg. The rate of blood flow to the preparation was 3 to 10 ml/min. The venous effluent from the preparation was led to a collecting funnel and returned to the support dog through the external jugular vein. The

preparation was anchored to a stainless steel bar and placed in a cup-shaped glass container kept at 37°C. The upper part of the cardiac preparation was connected to a force-displacement transducer (Nihon Kohden, Tokyo) by a silk thread. The cardiac tissue was usually stretched to a resting tension of 2 g. Isometric tension was recorded on a thermo-writing rectigraph (Nihon Kohden). A pair of bipolar silver electrodes was brought into contact with the epicardial surface of the isolated preparation in order to record the atrial electrogram or to drive the left ventricle electrically. The atrial rate was derived from the electrogram with a cardio-tachometer (Nihon Kohden). The femoral arterial blood pressure of the support dog, heart rate derived from lead II of the ECG and the rate of blood flow to an atrial or a left ventricular preparation

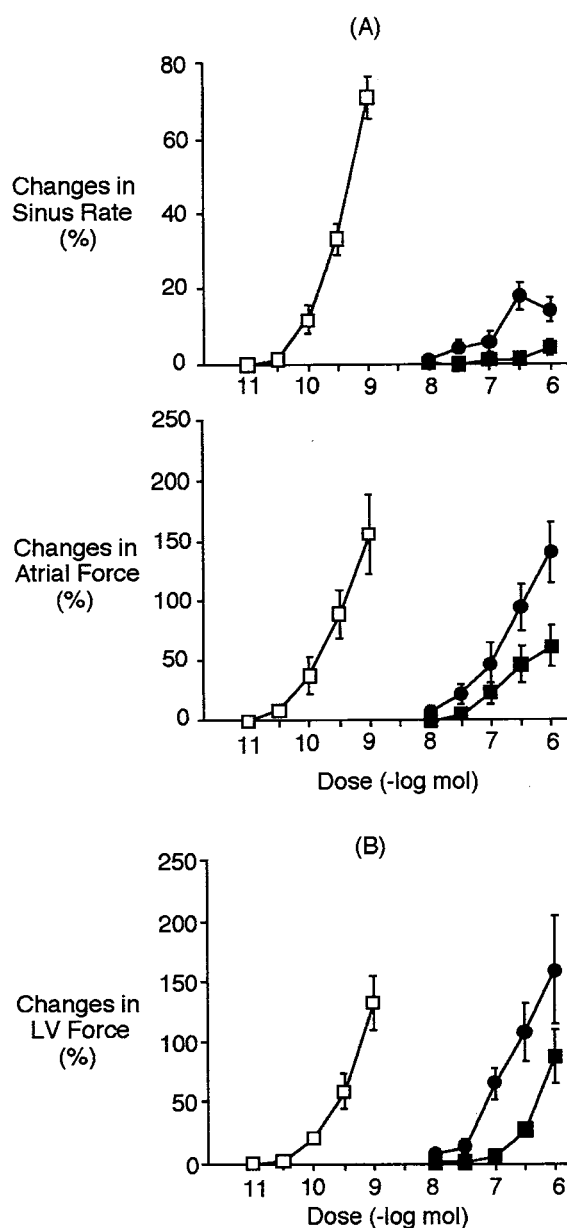


Fig. 2. Dose-response curves for percentage changes in chronotropic and inotropic responses in atrial (A) and ventricular (B) preparations to EMD 53998 (●), EMD 57033 (■) and norepinephrine (□). Points represent means and vertical bars show S.E.M. Basal levels of sinus rate and contractile force for seven isolated atria were  $116 \pm 6$  beats/min and  $3.0 \pm 0.5$  g, respectively, and the basal level of contractile force for five isolated ventricles was  $5.7 \pm 1.0$  g. NE, norepinephrine; LV, left ventricular.

were monitored simultaneously.

We examined the effects of EMD 57033 ( $0.01-1 \mu\text{mol}$ ), EMD 53998 ( $0.01-1 \mu\text{mol}$ ) and norepinephrine ( $0.01-1 \text{ nmol}$ ) on the sinoatrial nodal pacemaker activity and atrial contractility in 7 isolated right atria and in 5 isolated left ventricles. We also studied the effects of a  $\beta$ -adrenoceptor blocker, propranolol ( $10 \text{ nmol}$ ), on the

Table 1. Doses for the 15% increase ( $\text{ED}_{15}$ ) in sinus rate, 50% increase ( $\text{ED}_{50}$ ) in atrial force and 50% increase in ventricular force in response to EMD 53998, EMD 57033 and norepinephrine in the isolated, blood-perfused atrial and ventricular preparations of the dogs

Substance	Effective dose (nmol)		
	$\text{ED}_{15}$ of sinus rate	$\text{ED}_{50}$ of atrial force	$\text{ED}_{50}$ of ventricular force
Norepinephrine	$0.14 \pm 0.02$	$0.20 \pm 0.06$ (0.7)	$0.34 \pm 0.12$ (0.4)
EMD 53998	$231 \pm 54$	$191 \pm 64$ (1.2)	$103 \pm 20$ (2.2)
EMD 57033	$> 1000$	420 ( $> 2.4$ )	480 ( $> 2.1$ )
Sulmazole*	780	200 (3.9)	280 (2.8)
Pimobendan*	800	170 (4.7)	210 (3.8)
MCI-154*	9.1	5.4 (1.7)	22.3 (0.4)

$\text{ED}_{15}$  and  $\text{ED}_{50}$  for norepinephrine ( $n=5$ ) and EMD 53998 ( $n=7$ ) were determined from each dose-response curve, but those for EMD 57033 were determined from the mean dose-response curves from 7 experiments because EMD 57033 at doses of  $1000 \text{ nmol}$  or less did not always induce 50% or more increases in atrial or ventricular force. Numbers in the parentheses present the ratios of the dose for the 15% increase in sinus rate to the dose for 50% increase in atrial or ventricular force induced by each substance. \*Data of sulmazole, pimobendan and MCI-154 were cited from the references 2 and 4.

positive cardiac responses to EMD 57033, EMD 53998 and norepinephrine.

Drugs used in the present experiments were EMD 53998, ( $\pm$ )-5-(1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydro-6-quinolyl)-6-methyl-3,6-dihydro-2H-1,3,4-thiadiazino-2-one and EMD 57033, the (+)-enantiomer of EMD 53998 (both generously donated by E. Merck, Dramstadt, Germany); norepinephrine hydrochloride (Sankyo, Tokyo) and propranolol hydrochloride (Sigma, St. Louis, MO, USA). EMD 53998 and EMD 57033 were dissolved in DMSO to a concentration of  $50 \text{ mmol/l}$ , and lower concentrations were obtained by diluting with 0.9% NaCl. Other drugs were dissolved in 0.9% NaCl before starting the experiment. The amounts of drug solution were  $1-30 \mu\text{l}$ .

All data were expressed as means  $\pm$  S.E.M. An analysis of variance with Bonferroni's test was used for the statistical analysis of multiple comparisons of the data, and the paired Student's *t*-test was used for comparison between two groups. *P* values of less than 0.05 were considered statistically significant.

When EMD 53998 or norepinephrine was administered into the sinus node artery of an isolated atrium, positive chronotropic and inotropic effects were induced in a dose-dependent manner (Fig. 1A). On the other hand, EMD 57033 increased contractile force dose-dependently but did not increase sinus rate clearly. Summarized data from 7 isolated atria are shown in Fig. 2A. EMD 53998 ( $0.01-1 \mu\text{mol}$ ), EMD 57033 ( $0.01-1 \mu\text{mol}$ ) and norepinephrine ( $0.01-1 \text{ nmol}$ ) dose-dependently increased atri-

al contractile force, and both EMD compounds at high doses increased basal resting tension transiently as shown in Fig. 1A. EMD 57033 did not increase the sinus rate significantly, but EMD 53998 and norepinephrine increased the sinus rate in a dose-related manner. EMD 57033 and EMD 53998 at a dose of 1  $\mu\text{mol}$  increased the sinus rate by  $4 \pm 2\%$  (not significant) and by  $14 \pm 3\%$  ( $P < 0.05$ ), respectively, in 7 isolated atria. When EMD 53998 (0.01–1  $\mu\text{mol}$ ), EMD 57033 (0.01–1  $\mu\text{mol}$ ) and norepinephrine (0.01–1 nmol) were administered into the anterior descending branch of the left coronary artery of an electrically driven left ventricle, each compound induced positive inotropic effects in a dose-dependent manner (Figs. 1B and 2B).

Propranolol did not affect the changes in sinus rate, contractile force and resting tension in response to EMD 57033 (1  $\mu\text{mol}$ ) or EMD 53998 (0.3  $\mu\text{mol}$ ) when it blocked ( $P < 0.05$ ) the positive chronotropic and inotropic responses to norepinephrine (0.3 nmol) in 5 isolated dog atria.

The present study demonstrated that EMD 57033 at 1  $\mu\text{mol}$  did not increase sinus rate significantly when it readily increased right atrial and left ventricular contractile force with a transient increase in resting tension in the isolated, blood-perfused atrial and ventricular preparations of the dog. On the other hand, EMD 53998 at 1  $\mu\text{mol}$  increased both sinus rate and myocardial contractile force. Our results, therefore, suggest that a  $\text{Ca}^{2+}$  sensitizer such as EMD 57033 increases myocardial contractile force without an increase in sinus rate in the dog heart. However, because a high dose of EMD 57033 inhibited phosphodiesterase (5), the study of the chronotropic effect of a  $\text{Ca}^{2+}$  sensitizer might be limited.

Previously, in the isolated dog atrial and ventricular preparations, we have presented that phosphodiesterase inhibitors increase both the sinus rate and myocardial contractile force (2). We have also reported the positive inotropic and chronotropic effects of  $\text{Ca}^{2+}$  sensitizers with a phosphodiesterase inhibitory property, sulmazole, pimobendan and MCI-154 (2, 4). As presented in Table 1, sulmazole and pimobendan, but not MCI-154, increased sinus rate much less than did catecholamines and phosphodiesterase inhibitors (2, 4). However, EMD 57033 at the used doses did not increase the sinus rate significantly in isolated atria. EMD 57033 did not increase the sinus rate in isolated guinea pig hearts (5) and in anesthetized dog hearts (11), but EMD 57439 and EMD 53998 increased the sinus rate significantly parallel with the action of the phosphodiesterase inhibition in isolated guinea pig hearts (5). Thus, because a  $\text{Ca}^{2+}$  sensitizer neither increases intracellular  $\text{Ca}^{2+}$  nor activates membrane ionic currents directly, it probably does not affect pacemaker activity obviously in the dog atrial preparation.

We confirmed that EMD 53998 and EMD 57033 increased myocardial contractile force in the isolated perfused dog heart preparations as previously reported (5–8). In the isolated left ventricle, the percentage changes in the positive inotropic effects of EMD 53998 and EMD 57033 were slightly higher, whereas percentage changes in the inotropic effects of norepinephrine in the isolated ventricle were slightly smaller than those in the isolated atrium. Chiba et al. (2) reported that phosphodiesterase inhibitors induced positive chronotropic and inotropic responses in the atrial and ventricular preparations and that the inotropic effects in the ventricle were less than those in the atrium. Therefore, it is suggested that EMD 53998 and higher doses of EMD 57033 increase the ventricular contractility more effectively than the atrial contractility in comparison with a  $\beta$ -adrenoceptor agonist and a phosphodiesterase inhibitor in the dog heart like pimobendan in the same preparation (2). These results may be explained by the properties of EMD 53998 and EMD 57033: they cause an increase in sensitivity of contractile proteins to calcium and a prolongation of duration of the contractility in addition to a phosphodiesterase inhibition. We observed that the positive cardiac responses to EMD 53998 were 2 to 3 times more potent than those by EMD 57033, which is similar to a previous report performed on single cardiac myocytes of guinea pig (8); and this may be at least partly due to the additive effects of its (–)-enantiomer EMD 57439 (phosphodiesterase III inhibitor).

In our study, EMD 53998 and EMD 57033 increased the resting tension briefly and transiently as reported previously (12). Hgashiyama et al. (13) also reported that EMD 57033 increased the left ventricular end diastolic pressure, although it at appropriate doses did not affect diastolic function (11). These results are in agreement with the prior studies showing that EMD 53998 causes a reduction in diastolic cell length of cardiac myocytes (7, 8). However, sulmazole, pimobendan and MCI-154 did not increase the resting tension clearly in the isolated canine heart preparations (2, 4). Thus, the  $\text{Ca}^{2+}$  sensitizing actions of EMD compounds may be different from those of other  $\text{Ca}^{2+}$  sensitizers.

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