A Novel Cardiotonic Agent SCH00013 Acts as a Ca\(^{2+}\) Sensitizer with No Chronotropic Activity in Mammalian Cardiac Muscle\(^1,2\)

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ABSTRACT

We investigated the inotropic effect of SCH00013 (4,5-dihydro-6-[2-hydroxy-2-(4-cyanophenyl)ethyl]-1,2,5,6-tetrahydropyrido-4-yl)pyridazin-3(2H)-one) on isolated dog and rabbit ventricular cardiomyocytes. SCH00013 elicited a positive inotropic effect in a concentration-dependent manner (10\(^{-6}\) to 10\(^{-4}\) M) in both species in the presence of bupranolol. The positive inotropic effects of 10\(^{-4}\) M SCH00013 on the dog and rabbit were 38% and 29% of the maximal response to isoproterenol. SCH00013 did not alter the rate of beating in isolated rabbit right atria. In indo-1 loaded rabbit ventricular cardiomyocytes, SCH00013 at 10\(^{-4}\) M increased the systolic cell shortening by 52% above the base-line value in association with an insignificant increase in the systolic fluorescence ratio by 15% above the control. SCH00013 shifted the relationship between the Ca\(^{2+}\) transients and cell shortening to the left as compared with that of elevation of [Ca\(^{2+}\)]\(_{o}\). In the dog and rabbit ventricular muscles, carbachol partially inhibited the positive inotropic effect of SCH00013. SCH00013 did not affect the positive inotropic effect of isoproterenol at 3 \(	imes\) 10\(^{-6}\) M, but enhanced it at 3 \(	imes\) 10\(^{-5}\) M. These results indicate that SCH00013 is a cardiotonic agent that primarily acts via an increase in myofibrillar Ca\(^{2+}\) sensitivity with a moderate contribution of the cAMP-dependent mechanism at higher concentrations. SCH00013 has no chronotropic activity. The pharmacological profile of SCH00013 implies that the compound may be a promising cardiotonic agent for the treatment of congestive heart failure.

Cardiac glycosides and catecholamines have been used as cardiotonic agents for the treatment of heart failure. Because these agents have disadvantages such as narrow safety margin and arrhythmogenicity due to Ca\(^{2+}\) overload, extensive efforts have been focused on development of novel cardiotonic agents to replace these classical agents in the treatment of heart failure (Farah et al., 1984). Amrinone, milrinone, olprinone, vesnarinone, pimobendan and denopamine have been developed in the course of such an effort (Endoh and Hori, 1993). Although it has been demonstrated that these agents are effective in improving quality of life of the patients with heart failure due to improvements of hemodynamic parameters and exercise capacity, some of them (e.g., amrinone, milrinone) failed to prolong the life-span of patients but rather shortened it even compared with placebo (Kinney et al., 1982; Likoff et al., 1984; Packer, 1989; Packer et al., 1991), and the effects of other agents are still controversial (e.g., vesnarinone) (OPC-8212 Multicenter Research Group, 1990; Feldman et al., 1993, 1997; Scherrer-Crosdile et al., 1997) or under clinical investigation (e.g., pimobendan and denopamine) (Kino et al., 1986; Takarada et al., 1987; Kubo et al., 1992; Sasayama et al., 1994).

Classical cardiotonic agents act by an increase in intracellular Ca\(^{2+}\) mobilization (Smith et al., 1984a, 1984b; Endoh, 1996). In the clinical setting, most patients with congestive heart failure are treated with digitalis and angiotensin-converting enzyme inhibitors, and/or diuretics, and newly developed cardiotonic agents have been administered to the patients, whose hemodynamic parameters did not respond favorably to the classical pharmacological agents. The fact that digitalis is ineffective might mean that an increase in intracellular Ca\(^{2+}\) ions is not able to improve the symptom and such a situation may suffer readily from further facilitation of Ca\(^{2+}\) mobilizing process. Because these newly de-
developed agents act primarily by an increase in intracellular Ca\(^{2+}\) mobilization, Ca\(^{2+}\) overload could easily occur to result in harmful effects on the patients with digitalis-resistant heart failure.

The positive inotropic effects of cardiotonic agents are achieved by an increase in intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)), by an increase in the sensitivity of contractile proteins to Ca\(^{2+}\) ions (termed Ca\(^{2+}\) sensitizers) or by combinations of the two mechanisms (Blinks and Endoh, 1986). Ca\(^{2+}\) sensitizers that act through the latter mechanism do not require an increase in activation energy that is consumed to increase intracellular Ca\(^{2+}\) mobilization and to remove Ca\(^{2+}\) ions (Suga, 1990), and are devoid of arrhythmogenicity and myocardial cell injury due to intracellular Ca\(^{2+}\) overload (Endoh, 1996). Therefore, interests in the development of novel Ca\(^{2+}\) sensitizers as cardiotonic agents have been increasing, but up to now no such agents that primarily act as Ca\(^{2+}\) sensitizers are available for the clinical application to the patients with chronic heart failure.

We have screened the mechanism of action of a series of pyridazinone derivatives and chosen SCH00013 (4,5-dihydro-6-[1-[2-hydroxy-2-(4-cyanophenyl)ethy]-1,2,5,6-tetrahydropyrido-4-yl]pyridazin-3(2H)-one; fig. 1) as a novel cardiotonic agent to be developed for the treatment of patients with congestive heart failure. Because the positive force-frequency relationship disappears or inverted in ventricular myocardium isolated from severe heart failure (Muller et al., 1992; Schwinger et al., 1994), we focused to develop cardiotonic agents with least positive chronotropic action. The preliminary experiments indicated that this compound produces a positive inotropic effect without chronotropic action. In this study, we carried out experiments to elucidate the pharmacological profile of cardiac action of this compound. For this purpose we investigated the inotropic effect of SCH00013 in dog and rabbit ventricular myocardium and in indo-1 loaded rabbit ventricular cardiomycocytes. The preliminary accounts of this study have been published as an abstract (Sugawara and Endoh, 1997).

**Methods**

**Isolation of ventricular trabeculae of the dog and papillary muscles and right atria of the rabbit.** Mongrel dogs of either sex (7–12 kg) and male Japanese White rabbits (1.8–2.2 kg) were anesthetized with sodium pentobarbital (40 mg/kg, i.v.) and given heparin (600 units/kg, i.v.). The heart was rapidly excised, mounted on a Langendorff apparatus and retrogradely perfused for ~1 min at perfusion pressure of 80 cm H\(_2\)O with HEPES-Tyrode solution containing (in mM): NaCl 136.5, KCl 5.4, MgCl\(_2\) 0.53, CaCl\(_2\) 1.2, NaH\(_2\)PO\(_4\) 0.33, glucose 5.0, HEPES 5.0; pH 7.4 (adjusted with NaOH). The solution was continuously gassed with 100% O\(_2\) at 37°C. The heart was then perfused with nominally Ca\(^{2+}\)-free HEPES-Tyrode solution for 5 min, followed by perfusion with recirculation of Ca\(^{2+}\)-free HEPES-Tyrode solution to which collagenase (0.6 mg/ml) and protease (0.1 mg/ml) had been added. After approximately 20 min, when the heart became homogeneously soft, the enzymes were washed out for 1 min by perfusion with HEPES-Tyrode solution containing 0.2 mM CaCl\(_2\). The ventricles were then removed, minced in HEPES-Tyrode solution containing 0.2 mM CaCl\(_2\), and filtered through a nylon mesh (200 μm). The myocytes were resuspended in a stepwise manner in HEPES-Tyrode solution containing 0.2, 0.4, and 0.8 mM CaCl\(_2\). The myocytes were finally resuspended in HEPES-Tyrode solution containing 1.2 mM CaCl\(_2\) and kept for 1 hr or longer at room temperature (24 to 26°C) before the loading with the acetoxyethyl ester form of the Ca\(^{2+}\) sensitive fluorescence probe indo-1 (indo-1-AM).

**Simultaneous measurements of cell length and Ca\(^{2+}\) transient.** Myocytes were loaded with Indo-1/AM and all the after steps were carried out at room temperature (24–26°C). The loading solution consisted of 10 μl of 1 mM Indo-1/AM, 40 μl DMSO, 90 μl fetal bovine serum, 10 μl of 20% pluronic F-127 (wt/wt in DMSO), and 1 ml HEPES-Tyrode solution. The loading solution described above was sonicated for 3 min and 1 ml of cell suspension was added to it. The myocytes were allowed to load with Indo-1/AM for 1 to 4 min and then centrifuged at 150 rpm for 1 min. The supernatant was discarded and the pellet was resuspended in HEPES-Tyrode solution. The myocytes were placed in a perfusion chamber on the stage of an inverted microscope (Diaphot TMD 300, Nikon, Tokyo, Japan) equipped for simultaneous recordings of cell length and indo-1 fluorescence. After 10 min, the myocytes were perfused at a rate of about 2 ml/min with bicarbonate buffer containing (in mM) NaCl 116.4, KCl 5.4, MgSO\(_4\) 0.81, CaCl\(_2\) 1.2, NaH\(_2\)PO\(_4\) 1.02, glucose 5.0, NaHCO\(_3\) 23.8. The buffer was continuously gassed with 95% O\(_2\)-5% CO\(_2\) (pH 7.4). Bipolar platinum electrodes placed in the perfusion chamber were used to stimulate the myocytes with square-wave pulses of 5 msec duration and a voltage of 0.5 to 0.7 V at 0.5 Hz.

Indo-1 fluorescence was excited with the light from a xenon lamp with wavelength of 355 nm, reflected by a 380 nm long-pass dichroic mirror, and detected by means of a fluorescence spectrophotometer (CAM-200, Japan Spectroscopic, Tokyo, Japan). Excitation light was

**Fig. 1.** Chemical structure of SCH00013 (4,5-dihydro-6-[1-[2-hydroxy-2-(4-cyanophenyl)ethy]-1,2,5,6-tetrahydropyrido-4-yl]pyridazin-3(2H)-one).
applied to the myocyte through a neutral density filter to minimize the photobleaching of indo-1. The emitted fluorescence was collected by an objective lens (CF Fluor DL40, Nikon) and after passing through the 380 nm long-pass dichroic mirror, it was first separated by a 580 nm long-pass dichroic mirror (Omega Optical, Brattleboro, VT). The fluorescence light was subsequently split by a 425 nm dichroic mirror to permit simultaneous measurements of both 405 nm and 500 nm wavelengths through band-pass filters, respectively, by use of two separate photomultiplier tubes. The fluorescence ratio (405 nm/500 nm) was then used as an index of [Ca\(^{2+}\)].

The cell length was monitored simultaneously with indo-1 fluorescence using red light (>620 nm) through the normal bright field illumination optics of the microscope. The bright field image of the cell was collected by an objective lens and first separated by a 580 nm long-pass dichroic mirror (Omega Optical). This image was projected onto a photodiode array (C6294–01, Hamamatsu Photonics, Hamamatsu, Japan) scanned at every 5 msec.

Cell length and indo-1 fluorescence data were acquired by use of a computer (Power Macintosh 8100/100AV, Apple Computer, Cupertino, CA) with an A/D converter (MP-100A, BIOPAC Systems, Santa Barbara, CA) and analyzed after the low-pass filtering (cutoff frequency of 25 Hz) and averaging of 5 successive signals.

**Drugs and chemicals.** The following drugs were used; SCH00013 (Zenyaku Kogyo Co. Ltd., Tokyo, Japan); (+)-bupranolol hydrochloride (Kaken Pharmaceutical Co. Ltd., Tokyo, Japan); (-)-isoproterenol hydrochloride, carbachol (carbamylcholine chloride), clonidine (Hoechst Marion Roussel, Raritan, NJ); (–)-isoproterenol hydrochloride, carbachol (carbamylcholine chloride), fetal bovine serum, pluronic F-127 and protease (type XIV) (Sigma Chemical Co., St. Louis, MO); collagenase (class II, Worthington Biochemical, Freehold, NJ); EMD 57033 ((±)-5-[1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydro-6-quinolyl]-6-methyl-3,6-dihydro-2H-1,3,4-thiadiazin-2-one; E. Merck, Darmstadt, Germany); indo-1/AM (Dojindo Laboratories, Kumamoto, Japan).

**Statistical analysis.** Experimental values are presented as mean ± S.E.M. Statistical analysis of the data was performed by one-way analysis of variance followed by application of the Bonferroni/Dunn method. A value of P < .05 was considered to indicate a statistically significant difference.

**Results**

**Inotropic effects of SCH00013 on isolated dog and rabbit ventricular muscles.** SCH00013 at concentrations of greater than 10\(^{-6}\) M elicited a positive inotropic effect on isolated dog ventricular trabeculae in a concentration-dependent manner (fig. 2A). The positive inotropic effect of SCH00013 at 10\(^{-4}\) M (the highest concentration examined because the compound produced insoluble precipitation at 3 × 10\(^{-4}\) M) was 43.3 ± 4.4% of ISOmax. Thus, the EC\(_{50}\) value of SCH00013 was approximately (1.60 ± 0.21) × 10\(^{-5}\) M or higher. The positive inotropic effect of SCH00013 was not influenced by 3 × 10\(^{-7}\) M of bupranolol (fig. 2A). The positive inotropic effect at 10\(^{-4}\) M and EC\(_{50}\) value of SCH00013 in the presence of bupranolol were 37.5 ± 3.9% of ISOmax and approximately (1.47 ± 0.38) × 10\(^{-5}\) M, which did not significantly differ from those in the absence of bupranolol. SCH00013 did not affect the time course of isometric contractions in dog ventricular trabeculae as shown in figure 2B.

![Fig. 2. Effects of SCH00013 on force of contraction and the time course of contraction in isolated dog ventricular trabeculae and rabbit ventricular papillary muscles which were electrically stimulated by square-wave pulses of 5 msec duration and a voltage ~20% above the threshold at 0.5 Hz and 1.0 Hz, respectively, through bipolar platinum electrodes. A and B, Concentration-response curves for the positive inotropic effect (A) and the time course of contraction (B) in dog ventricular trabeculae. The response to SCH00013 was determined in the absence or presence of 3 × 10\(^{-7}\) M of bupranolol. The positive inotropic response to SCH00013 in the presence of bupranolol in B was not significantly different from the response in its absence. Bupranol was administered in the organ bath 30 min before determination of the force of contraction and present throughout the experiments. Basal force of contraction: 11.9 ± 3.0 mN/mm\(^2\) (n = 7) in the control group and 16.3 ± 2.3 mN/mm\(^2\) (n = 7) in the bupranol group; ISOmax: 23.6 ± 3.1 mN/mm\(^2\) in the control group and 30.7 ± 4.3 mN/mm\(^2\) in the bupranol group. C and D, Concentration-response curves for the positive inotropic effect (C) and the time course of contraction (D) in the presence of 3 × 10\(^{-7}\) M bupranol in rabbit right ventricular papillary muscles. Basal force of contraction: 6.8 ± 1.2 mN/mm\(^2\) (n = 10); ISOmax: 17.6 ± 1.7 mN/mm\(^2\); b: the base-line levels before administration of SCH00013 were assigned to zero. Asterisks indicate the threshold concentration (+P < .05 vs. the corresponding base-line values).
In isolated rabbit papillary muscles, SCH00013 elicited a positive inotropic effect over an identical concentration range as seen in the dog. At concentrations of greater than $10^{-6}\text{ M}$ SCH00013 elicited a positive inotropic effect in a concentration-dependent manner on isolated rabbit papillary muscles in the presence of $3 \times 10^{-7}\text{ M}$ bupranolol (fig. 2C). The positive inotropic effect of SCH00013 at $10^{-4}\text{ M}$ was 29.4 ± 5.5% of ISOmax. The EC$_{50}$ value of SCH00013 was approximately (8.96 ± 2.36) $\times 10^{-6}\text{ M}$ or higher. SCH00013 prolonged the isometric contractions in rabbit papillary muscles in the presence of bupranolol. The duration of contraction and relaxation time were significantly increased, whereas time to peak force was not affected (fig. 2D). Thus, SCH00013 prolonged the duration of contraction, primarily by prolongation of relaxation time in the rabbit papillary muscle.

**Chronotropic effects of SCH00013 on isolated rabbit right atria.** The effect of SCH00013 on the rate of beating in isolated rabbit right atria is shown in table 1. SCH00013 (3 $\times 10^{-7}$ to $10^{-4}\text{ M}$) did not significantly alter the rate of beating. SCH00013 on cell shortening and Ca$^{2+}$ transients in rabbit ventricular cardiomyocytes. Representative tracings of the effects of elevation of extracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_o$) isoproterenol, SCH00013 and EMD 57033 on cell shortening and indo-1 fluorescence ratio in indo-1 loaded rabbit ventricular cardiomyocytes are shown in figures 3 and 4, respectively. Elevation of [Ca$^{2+}]_o$ increased cell shortening in a concentration-dependent manner in association with increases in indo-1 fluorescence ratio (fig. 3A). Ca$^{2+}$ transients were slightly abbreviated by an elevation of [Ca$^{2+}]_o$, but the duration of cell shortening was scarcely affected (fig. 3A, i). Isoproterenol, a beta adrenoceptor agonist, also exerted a concentration-dependent positive inotropic effect, accompanied by a pronounced increase in indo-1 fluorescence ratio (fig. 3B). Isoproterenol markedly abbreviated the duration of cell shortening and indo-1 fluorescence ratio (fig. 3B, h). By contrast, SCH00013 and EMD 57033, Ca$^{2+}$ sensitizers, increased cell shortening with little changes in the amplitude of indo-1 fluorescence ratio (fig. 4, A and B). The time course of cell shortening and indo-1 fluorescence ratio was scarcely affected by SCH00013 (fig. 4A, h). The diastolic cell length was affected less by SCH00013 than by EMD 57033: the diastolic cell length was somewhat reduced by SCH00013 at concentrations higher than $3 \times 10^{-5}\text{ M}$ (fig. 4A), though not as strongly as with EMD 57033 (fig. 4B). On the other hand, the positive inotropic effect of EMD 57033 was accompanied by a striking reduction in diastolic cell length (fig. 4B). Moreover, EMD 57033 prolonged the time course of beating with little changes in the time course of indo-1 fluorescence ratio (fig. 4B, g).

The summarized data are presented in figure 5. Elevation of [Ca$^{2+}]_o$ and isoproterenol increased both systolic levels of cell length and fluorescence ratio in a concentration-dependent manner (fig. 5, A and B). The systolic levels of cell length and fluorescence ratio were increased by [Ca$^{2+}]_o$, at 14.4 mM to 272.6 ± 39.9% and 206.7 ± 10.9% of the control values, respectively. Isoproterenol at $3 \times 10^{-6}\text{ M}$ increased the systolic levels of cell length to 241.5 ± 28.6% and fluorescence ratio to 227.9 ± 14.4% of the control values. In contrast to elevation of [Ca$^{2+}]_o$ and isoproterenol, neither SCH00013 nor EMD 57033 increased the systolic level of fluorescence ratio, when these compounds increased systolic cell shortening (fig. 5, C and D). The systolic cell shortening was significantly increased to 152.1 ± 2.2% of the control value with SCH00013 at $10^{-4}\text{ M}$ without a significant change in systolic level of fluorescence ratio (114.8 ± 6.7% of the control value; P > .05). The systolic cell shortening was significantly increased by EMD 57033 at $3 \times 10^{-6}\text{ M}$ to 233.7 ± 16.4% of the control value with no change in the systolic level of fluorescence ratio (100.0 ± 8.5% of the control value; P > .05).

Figure 6 shows the relationship between the systolic levels of indo-1 fluorescence ratio and systolic cell shortening during exposure to increasing concentrations of [Ca$^{2+}]_o$, isoproterenol, SCH00013 and EMD 57033. SCH00013 and EMD 57033 shifted the relationship to the left as compared with those of elevation of [Ca$^{2+}]_o$, and isoproterenol. Isoproterenol at $10^{-6}\text{ M}$ and higher shifted the relationship to the right as compared with that of elevation of [Ca$^{2+}]_o$.

**Effects of carbachol on the positive inotropic effect of SCH00013.** Carbachol inhibits selectively the cAMP-dependent positive inotropic effect of cardiotonic agents in mammalian ventricular myocardium (Endoh, 1980, 1987). Therefore, we investigated whether cAMP would be involved in the positive inotropic effect of SCH00013 by use of carbachol in isolated dog and rabbit ventricular muscles.

Carbachol ($3 \times 10^{-6}\text{ M}$) shifted the concentration-response curves for SCH00013 to the right and downward, but did not abolish the positive inotropic effect of SCH00013, as shown in figure 7, A (dog) and B (rabbit). The extent of inhibition induced by carbachol was more pronounced at higher concentrations of SCH00013, indicating that the contribution of the cAMP-dependent effect is increased when the concentration of SCH00013 increases. In the dog ventricular trabeculae, the response to SCH00013 at $10^{-4}\text{ M}$ was decreased by carbachol from 37.5% to 15.7% of ISOmax (fig. 7A). In the rabbit papillary muscle, the response to SCH00013 at $10^{-4}\text{ M}$ was decreased by carbachol from 29.4% to 12.5% of ISOmax (fig. 7B). It was noted that the force of contraction in the presence of carbachol was still significantly greater than the basal force in both species. These results suggest a significant contribution of the cAMP-dependent mechanism to the positive inotropic effect of SCH00013, namely at higher concentrations.

**Effects of SCH00013 on the positive inotropic effect of isoproterenol.** The influence of SCH00013 on the concentration-response curves for the positive inotropic effect of isoproterenol in dog ventricular trabeculae and rabbit papillary muscles is shown in figure 8. In both species, SCH00013 at $3 \times 10^{-6}\text{ M}$ and $3 \times 10^{-5}\text{ M}$ elicited a positive inotropic effect in a concentration-dependent manner (fig. 8, A and C).

### Table 1

<table>
<thead>
<tr>
<th>SCH00013</th>
<th>Rate of beating</th>
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<tr>
<td>M</td>
<td>beats/min</td>
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<tr>
<td>0</td>
<td>183.0 ± 15.6</td>
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<tr>
<td>$3 \times 10^{-7}$</td>
<td>182.1 ± 15.5</td>
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<tr>
<td>$10^{-6}$</td>
<td>180.4 ± 15.5</td>
</tr>
<tr>
<td>$3 \times 10^{-6}$</td>
<td>174.9 ± 14.6</td>
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<td>$10^{-5}$</td>
<td>171.4 ± 13.0</td>
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<tr>
<td>$3 \times 10^{-5}$</td>
<td>177.4 ± 12.6</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>185.7 ± 12.1</td>
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The concentration-response curve for isoproterenol was determined in the absence or in the presence of different concentrations of SCH00013. In both species, the concentration-response curve for isoproterenol is not affected by SCH00013 at 3 \times 10^{-6} \text{ M}, but it appears to be shifted to the left and upward by SCH00013 at 3 \times 10^{-5} \text{ M} (fig. 8, A and C). The increase induced by SCH00013 was subtracted from the total increase \([\text{isoproterenol} + \text{SCH00013}] \) minus SCH00013, and the concentration-response curve for isoproterenol was constructed with the remaining increase caused by isoproterenol expressed as 100% and compared with the curve with isoproterenol alone: the concentration-response curve for isoproterenol was shifted to the left in a parallel manner in both species (fig. 8, B and D). In the dog ventricular trabeculae, the pD2 values for isoproterenol in the presence of 3 \times 10^{-6} \text{ M} and 3 \times 10^{-5} \text{ M} SCH00013 were 7.70 and 8.26, respectively. The value in the presence of 3 \times 10^{-5} \text{ M} SCH00013 was significantly higher than the control value of 7.53. In the rabbit papillary muscles, the pD2 value in the presence of 3 \times 10^{-5} \text{ M} SCH00013 (8.94) was also significantly greater than the control value (8.46).

**Discussion**

The pyridazinone derivative SCH00013 elicited a concentration-dependent positive inotropic effect in isolated dog and rabbit ventricular myocardium. The positive inotropic effect of SCH00013 was not affected by bupranolol, a potent beta adrenoceptor blocking agent, implying that the activation of beta adrenoceptors is not involved in the positive inotropic effect of the compound. The characteristic pharmacological profile of SCH00013 is that it elicits a positive inotropic effect (fig. 2) without chronotropic effect (table 1). The present findings indicate that two mechanisms, the increase in Ca^{2+} sensitivity of contractile proteins, which may be independent of cAMP metabolism, and the cAMP-dependent regulation that may involve the inhibition of cAMP PDE, contribute to the positive inotropic effect of SCH00013.
An increase in myofibrillar Ca\(^{++}\) sensitivity by SCH00013. In isolated rabbit ventricular cardiomyocytes loaded with indo-1/AM, elevation of [Ca\(^{++}\)]\(_{o}\) and isoproterenol increased the extent of cell shortening in association with an increase in the amplitude of Ca\(^{++}\) transients in a concentration-dependent manner (fig. 3). By contrast, EMD 57033, a prototype Ca\(^{++}\) sensitizer (Solaro et al., 1993), and SCH00013 increased the cell shortening with little increase in the amplitude of Ca\(^{++}\) transients (fig. 4). The experimental system used to detect the inotropic effect of drugs is not ideal because the cell is not stretched to the optimal length in Frank-Starling mechanism. Nevertheless, the relationship between the peak fluorescence ratio and the systolic cell shortening was shifted to the right by isoproterenol and to the left by EMD 57033, an indication that the experimental system reflects well the qualitative modulation of myofibrillar Ca\(^{++}\) sensitivity (fig. 6) as also shown in the previous study (Fujita and Endoh, 1996). While the mechanism for the increase in myofibrillar Ca\(^{++}\) sensitivity induced by SCH00013 is not clear, the differences in the effects of the compound from those of EMD 57033 are evident from the present findings as follows: 1) the positive inotropic effect of SCH00013 is more moderate than that of EMD 57033; 2) SCH00013 affected the time course of neither Ca\(^{++}\) transients nor cell shortening, while EMD 57033 increased the duration of cell shortening without affecting the duration of Ca\(^{++}\) transients; and 3) SCH00013 had only a slight effect on the diastolic cell length, while EMD 57033 decreased markedly the diastolic level of cell length at concentrations of 10\(^{-6}\) M and higher (fig. 4). The last difference is considered to be of importance with respect to the potential risk of diastolic dysfunction that might occur in clinical application of certain Ca\(^{++}\) sensitizers especially to the patients with severe congestive heart failure (Hajjar et al., 1997). Although Ca\(^{++}\) sensitizers have a favorable effect on myocardial energy consumption, impairment of cardiac relaxation is a possible adverse effect of Ca\(^{++}\) sensitizers (Higashiyama et al., 1995; Nielsen-Kudsk and Aldershvile, 1995; Hajjar et al., 1997). In

Fig. 4. Representative tracings of the effects of SCH00013 (A) and EMD 57033 (B) on cell shortening and Ca\(^{++}\) transients in indo-1 loaded rabbit ventricular cardiomyocytes. A, The myocyte was exposed to increasing concentrations of SCH00013 that were indicated by horizontal bars in upper panel; a, control before addition of SCH00013; g, after washout of SCH00013; h, amplitudes of tracings in a and f have been adjusted electronically and superimposed to facilitate comparison of their time courses; a–g in bottom are recorded at times corresponding to a–g in top. B, The myocyte was exposed to increasing concentrations of EMD 57033 that were indicated by horizontal bars in top; a, control before addition of EMD 57033; f, after washout of EMD 57033; g, amplitudes of tracings in a and e have been adjusted electronically and superimposed to facilitate comparison of their time courses; a–f in bottom are recorded at times corresponding to a–f in top.
fact, EMD 57033 reduced diastolic cell length and prolonged relaxation time of cell shortening with no changes in Ca\textsuperscript{2+} transients in the rabbit ventricular cardiomyocyte (fig. 4B).

cAMP-mediated effects of SCH00013. The absence of effects of SCH00013 to shorten the diastolic cell length may be partly due to contribution of cAMP-mediated effect of SCH00013. The following pieces of evidence indicate the involvement of cAMP in the effect of SCH00013: 1) the positive inotropic effect of SCH00013 was suppressed by carbachol (fig. 7); and 2) SCH00013 at $3 \times 10^{-5}$ M shifted the concentration-response curve for isoproterenol to the left (fig. 8). A muscarinic receptor antagonist, carbachol, does not affect the basal force of contraction and cAMP-independent positive inotropic effect of agents such as ouabain, elevation of [Ca\textsuperscript{2+}]\textsubscript{i}, and alpha adrenoceptor agonists, but suppresses selectively the cAMP-mediated effect of agents such as iso-

Fig. 5. Effects of [Ca\textsuperscript{2+}], (A), isoproterenol (B), SCH00013 (C) and EMD 57033 (D) on systolic levels of cell shortening and fluorescence ratio of indo-1 loaded in rabbit ventricular cardiomyocytes. Only one concentration-response relationship was determined in each cardiomyocyte. Diastolic cell lengths and extents of shortening in the base line before respective interventions: 138.2 $\pm$ 6.6 $\mu$m and 7.58 $\pm$ 0.94% in A ($n = 7$); 140.8 $\pm$ 7.8 $\mu$m and 7.04 $\pm$ 0.55% in B ($n = 7$); 152.4 $\pm$ 7.9 $\mu$m and 7.73 $\pm$ 0.31% in C ($n = 10$); 140.3 $\pm$ 7.7 $\mu$m and 8.07 $\pm$ 0.93% in D ($n = 7$). Numbers in parentheses in A and B indicate the number of myocytes at highest concentrations, at which two myocytes, respectively, became arrhythmic and were therefore deleted from the analysis. Asterisks indicate the threshold concentration ($*P < .05$ vs. the corresponding control values).

Fig. 6. The relationship between the systolic levels of cell length and fluorescence ratio during exposure to increasing concentrations of [Ca\textsuperscript{2+}], isoproterenol, SCH00013 and EMD 57033 in indo-1 loaded rabbit ventricular cardiomyocytes. Data are taken from those in figure 5.

Fig. 7. Influence of carbachol on the positive inotropic effect of SCH00013 in isolated dog ventricular trabeculae (A) and rabbit right ventricular papillary muscles (B) which were electrically stimulated by square-wave pulses of 5 msec duration and a voltage about 20% above the threshold at 0.5 Hz and 1.0 Hz, respectively, through bipolar platinum electrodes. The response to SCH00013 was determined in the presence of $3 \times 10^{-7}$ M bupranolol. Only one concentration-response curve was determined in each preparation. The control concentration-response curves are the same as those presented in figure 2; b: the base-line levels before administration of SCH00013 were assigned to zero. In the carbachol group, the basal force of contraction was 13.6 $\pm$ 4.2 mN/mm\textsuperscript{2} in the dog ($n = 7$) and 5.3 $\pm$ 1.7 mN/mm\textsuperscript{2} in the rabbit ($n = 8$). ISOmax was 19.7 $\pm$ 3.4 mN/mm\textsuperscript{2} in the dog and 14.3 $\pm$ 3.1 mN/mm\textsuperscript{2} in the rabbit. Asterisks indicate the threshold concentration ($*P < .05$ vs. the corresponding base-line values).
Fig. 8. Influence of SCH00013 on the positive inotropic effect of isoproterenol in isolated dog ventricular trabeculae (A and B) and rabbit ventricular papillary muscles (C and D) which were electrically stimulated by square-wave pulses of 5 msec duration and a voltage about 20% above the threshold at 0.5 Hz and 1.0 Hz, respectively, through bipolar platinum electrodes. Only one concentration-response curve for isoproterenol was determined in each preparation. A and C, Concentration-response curves for isoproterenol are constructed with isoproterenol alone (control, ○), isoproterenol + 3 × 10⁻⁶ M SCH00013 (△), and isoproterenol + 3 × 10⁻⁶ M SCH00013 minus SCH00013 (◆), respectively; b: the base-line levels before administration of isoproterenol. Basal force of contraction in A: 9.6 ± 0.7 mN/mm² in control group (n = 5) and 11.3 ± 1.1 mN/mm² in group with 3 × 10⁻⁶ M SCH00013 (n = 7), 13.7 ± 2.3 mN/mm² in group with 3 × 10⁻⁶ M SCH00013 minus SCH00013 (◆) and isoproterenol + 3 × 10⁻⁶ M SCH00013 minus SCH00013 (△), respectively; b: the base-line levels before administration of isoproterenol. Basal force of contraction in C: 7.4 ± 0.6 mN/mm² (n = 5) and 9.7 ± 2.5 mN/mm² in group with 3 × 10⁻⁶ M SCH00013 (n = 5) and 9.7 ± 2.5 mN/mm² in group with 3 × 10⁻⁶ M SCH00013 (n = 4). The corresponding ISOmax values were 37.0 ± 5.6, 25.4 ± 9.1 and 25.7 ± 6.0 mN/mm² (n = 4 or 5), respectively. *P < .05 vs. the corresponding control values.

proterenol, papaverine and theophylline in dog ventricular myocardium (Endoh, 1979, 1980, 1987). Carbachol inhibited the positive inotropic effect of SCH00013 by about 50% in both dog and rabbit ventricular muscle (fig. 7). These results imply that the positive inotropic effect of SCH00013 namely at higher concentrations may be due to an approximately equal contribution of cAMP-mediated and cAMP-independent mechanism (an increase in myofibrillar Ca²⁺ sensitiv-ity).

The inhibitory action of SCH00013 on the cAMP PDE may be responsible for the cAMP-mediated regulation because SCH00013 (3 × 10⁻⁵ M) enhanced the positive inotropic effect of isoproterenol (fig. 8). SCH00013 inhibits the activity of PDE III isolated from guinea pig heart with an IC₅₀ value of 7.3 × 10⁻⁵ M (unpublished data). It is noteworthy, however, that in the isolated rabbit papillary muscle SCH00013 at 3 × 10⁻⁶ M that produced a definite positive inotropic effect (fig. 2) did not enhance the effect of isoproterenol and first at 3 × 10⁻⁵ M it shifted the concentration-response curve for isoproterenol to the left (fig. 8). These observations indicate that SCH00013 is not potent as a cAMP PDE inhibitor and the magnitude of contribution of cAMP-independent effect namely at low concentrations may be greater than that of the cAMP-dependent effect. The findings that carbachol suppressed the positive inotropic effect of SCH00013 more markedly over higher than lower concentration range (fig. 7) support a greater contribution of cAMP at higher concentra-
tions. SCH00013 was slightly less potent in single myocytes (figs. 4 and 5) than in isolated papillary muscles (fig. 2) of the rabbit in inducing the positive inotropic effect. This may be mainly due to the difference in experimental conditions: pap-
illary muscles contract in an isometric manner at 1 Hz being stretched to near Lmax at 37°C, while single myocytes con-
tract in an isotonic (auxotonic) manner from the slack length at room temperature.

Overall cardiac effects of SCH00013. The duration of isometric contractions of the muscle was not abbreviated, but was unchanged (dog) or rather prolonged (rabbit) by SCH00013 (fig. 2). These findings contradict to the contribu-
tion of cAMP-mediated effect to the myocardial contractility in general, which is characterized by a pronounced abbrevi-
ation of duration of contraction and acceleration of relaxation (Endoh and Blinks, 1988; isoproterenol in fig. 3). This may be partly due to a balance between cAMP-mediated and Ca²⁺ sensitizing action.

Another characteristic property of SCH00013 is a lack of positive chronotropic effect, which is due to screening of the compound that has no or less positive chronotropic action. However, the cellular mechanism by which no positive chronotropic effect of the agent that facilitates a cAMP-mediated process can occur remains obscure. In this respect, the profile of SCH00013 has a close resemblance to that of vesnarinone in which the cAMP-mediated positive chronotropic effect is counteracted by its effect to decrease the K⁺ conductance in
myocardial cell membrane (Iijima and Taira, 1987). The observations that SCH00013 prolonged the duration of contraction in isolated rabbit papillary muscles (fig. 2) support such a possibility that the compound prolongs the duration of action potential by inhibition of K⁺ channels. No matter how the mechanism involved, the lack of positive chronotropic effect may be beneficial for the application to the patients with congestive heart failure because of the avoidance of unnecessary increase in oxygen consumption, which is an important determinant to exacerbate the heart failure syndrome, and because of disappearance or inversion of the positive force-frequency relationship in these patients (Muleri et al., 1992; Schwingler et al., 1994).

In conclusion, SCH00013 produces a positive inotropic effect predominantly through myofibrillar Ca⁺⁺ sensitization with a moderate contribution of cAMP-dependent mechanism. It does not alter the heart rate. These observations imply that SCH00013 may have potential as a cardiotoxic agent with novel mechanisms of action for the treatment of congestive heart failure.

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