NOTE *Pharmacology*

Endostatin inhibits T-type Ca^{2+} channel current in guinea pig ventricular myocyte

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ABSTRACT. Endostatin, a fragment of collagen XVIII, is known as an endogenous angiogenesis inhibitor, and its serum concentration increases in various cardiovascular diseases. T-type Ca^{2+} channel, low voltage-activated Ca^{2+} channel, is not expressed in adult ventricular myocytes. Re-expression of T-type Ca^{2+} channels in cardiac myocytes is thought to be involved in the development of cardiac hypertrophy. We examined the effects of endostatin on T-type Ca^{2+} channel current by whole-cell patch clamp technique in freshly isolated adult guinea pig ventricular myocytes, which exceptionally express T-type Ca^{2+} channels. Although endostatin 300 *n*g/m*l* had no effect on L-type Ca^{2+} current, it significantly inhibited T-type Ca^{2+} current. These data indicate that endostatin can be an endogenous inhibitor of T-type Ca^{2+} channels in the cardiac myocytes.

KEY WORDS: endostatin, guinea pig ventricular myocyte, T-type Ca^{2+} channel

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Endostatin, a non-collagenous C-terminal fragment cleaved from collagen XVIII, is an endogenous angiogenesis inhibitor [13]. A number of clinical trials of endostatin have been conducted for a new anti-cancer therapy development. Recent studies have revealed that the serum concentration and the expression levels of endostatin increased in various cardiovascular diseases, such as cardiac hypertrophy, myocardial infarction and heart failure [5, 8]. Therefore, endostatin might be involved in the development of cardiac diseases. However, the effect of endostatin on heart has not been clarified.

In cardiac myocytes, two types of voltage-gated Ca^{2+} channels are expressed in the cell membrane [9]. High-voltage-activated L-type Ca^{2+} channels are responsible for the main Ca^{2+} entry into the cardiac myocytes associated with the depolarization, which triggers the cardiac contraction [16]. On the contrary, T-type Ca^{2+} channels are low-voltage-activated Ca^{2+} channels and open after small depolarization of the membrane. In the adult mammal heart, T-type Ca^{2+} channels are found in the pacemaker cells located in sino-atrial node [1]. T-type Ca^{2+} channels are also expressed in the embryonic ventricular myocytes and are involved in the development of the cells [19]. Many studies demonstrated that T-type Ca^{2+} channels were absent in the adult ventricular myocytes of rats, cats and dogs [15]. Although T-type Ca^{2+} channels disappear during maturation of cardiac myocytes, re-expression of T-type Ca^{2+} channels was observed in the cardiac myocytes of mice, rats and cats with pathological conditions, such as cardiac hypertrophy and post-myocardial infarction [4, 11, 15]. Re-expression of T-type Ca^{2+} channels is thought to play an important role in the enlargement of cardiac myocytes [3]. It has been reported that endostatin inhibits cell proliferation and migration through the inhibition of T-type Ca^{2+} channels in human glioblastoma cells [20]. However, the influence of endostatin on voltage-gated Ca^{2+} channels in cardiac myocytes has not been clarified. Therefore, we investigated the effect of endostatin on T-type Ca^{2+} channel current by using whole-cell patch clamp technique in the adult guinea pig ventricular myocytes, which exceptionally express T-type Ca^{2+} channels [12].

The present study was conducted in accordance with the Guide for Care and Use of Laboratory Animals as adopted by Kitasato University. Isolation of ventricular myocytes was performed as described previously [14]. Briefly, the heart of adult guinea pig (248–602 g body weight) was isolated under sodium pentobarbital anesthesia (70 mg/kg, i.p.) and perfused by a modified Langendorff apparatus. The perfused heart was digested by 0.02% collagenase, and ventricular myocytes were isolated. Electrophysiological recording of membrane currents was performed by whole-cell patch clamp method as described previously [7]. The external solution contained the following compositions: 137 mM Tris, 1 mM $MgCl_2$, 5.4 mM $CaCl_2$, 20 mM CsCl and 5 mM glucose adjusted to pH of 7.4 with HCl. The pipette solution contained the following compositions: 125 mM CsOH, 5 mM ATP-Mg, 15 mM EGTA, 20 mM TEA-Cl and 10 mM HEPES adjusted to pH 7.2 with CsOH [21]. Ca^{2+} currents were elicited by 300 ms depolarizing test pulses from a holding potential (HP) of −90 and −50 mV. Whole Ca^{2+} channel current (I_{Ca}) was elicited by 15 steps depolarizing pulses of each +10 mV from a HP of −90 mV. L-type Ca^{2+} channel current (I_{CaL}) was elicited by 11 steps depolarizing pulses of each +10 mV from a HP of −50 mV [17]. The current density (pA/pF) was calculated by normalizing current amplitude by cellular membrane capacitance.

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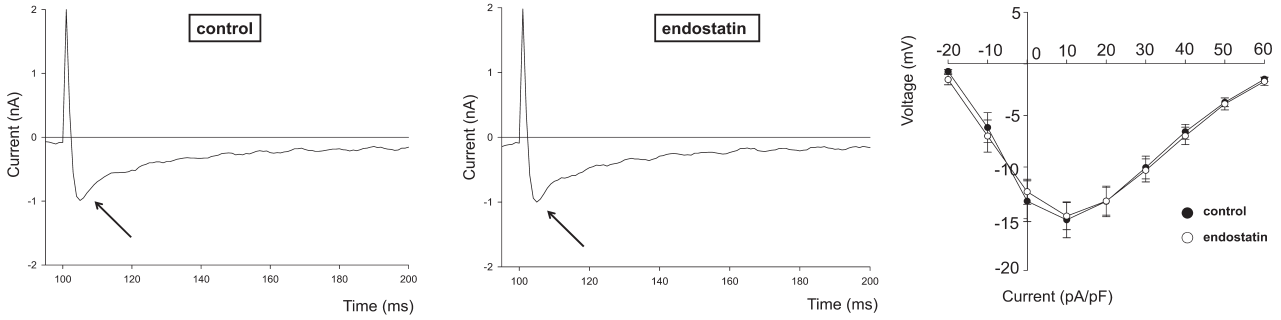

Fig. 1. Endostatin had no effect on L-type Ca^{2+} channel current in isolated guinea pig ventricular myocytes. Representative L-type Ca^{2+} channel current (I_{CaL}) records (arrows; holding potential: −40 mV) at 10 mV in the same cell are shown (control: left, endostatin: middle). Current-Voltage relations for the peak currents of I_{CaL} (−20–60 mV) in the absence (closed symbols) or presence (open symbols) of endostatin (300 *n*g/m*l*, 5 min-pretreatment) are shown (right). The current density (pA/pF) was calculated by normalizing current amplitude by cellular membrane capacitance. Results were expressed as means ± S.E.M (n=11).

Results are expressed as mean ± S.E.M. Statistical analysis was performed by Student's *t*-test. A value of $P<0.05$ was considered as statistically different.

T-type Ca^{2+} channel current (I_{CaT}) was obtained by subtracting I_{CaL} from I_{Ca}. In the present study, the peak inward current of I_{CaT} was observed at −20 mV. It was consistent with the previous report [21]. Lee *et al*. reported that $NiCl_2$ inhibited I_{CaT} in guinea pig ventricular myocyte [10]. This study confirmed that $NiCl_2$ 100 μM significantly inhibited the I_{CaT} (Fig. 2A, at −20 mV, control: −1.15 ± 0.20 pA/pF, $NiCl_2$: −0.15 ± 0.17 pA/pF, n=4). First, we investigated the effect of endostatin on I_{CaL}. Endostatin (300 *n*g/m*l*) was bath applied 5 min before the stimulation of depolarizing pulse. It was reported that blood concentration of endostatin in healthy volunteers is about 40 *n*g/m*l* [18]. On the other hand, the concentration in patients of cardiovascular disease reached about 250–300 *n*g/m*l* [6]. Therefore, the concentration of endostatin 300 *n*g/m*l* was within the pathological range. In the present study, endostatin 300 *n*g/m*l* had no effect on I_{CaL} (Fig. 1, at +10 mV, control: −15.2 ± 2.0 pA/pF, endostatin: −14.8 ± 1.5 pA/pF, n=11). Zhang *et al*. reported that endostatin had no effect on I_{CaL} in glioblastoma cells [20]. Our results were consistent with this report. Next, we investigated the effect of endostatin on I_{CaT}. Endostatin 300 *n*g/m*l* significantly inhibited I_{CaT} at −20 mV (Fig. 2B, control: −1.18 ± 0.12 pA/pF, endostatin: −0.75 ± 0.10 pA/pF, n=11, $P<0.05$), while endostatin 300 *n*g/m*l* did not inhibit it at −30 mV (Fig. 2, control: −0.99 ± 0.12 pA/pF, endostatin: −0.90 ± 0.10 pA/pF, n=11) and −40 mV (Fig. 2, control: −0.66 ± 0.11 pA/pF, endostatin: −0.62 ± 0.10 pA/pF, n=11). Bladen and Zamponi reported that a $Na_v1.8$ blocker 5-(4-chlorophenyl-*N* (3, 5-dimethoxyphenyl) furan-2-carboxamide (A803467) inhibited T-type Ca^{2+} channel in tsA-201 cells. Hyperpolarizing shift of both half-activation voltage and peak current of I_{CaT} was observed in A803467-treated cells [2]. We suppose that similar hyperpolarizing shift of peak current of I_{CaT} might be occurred in endostatin-treated cardiomyocytes, which would blunt the inhibition of I_{CaT} at −30 and −40 mV. We confirmed that low dose endostatin (30 *n*g/m*l*) did not significantly inhibit I_{CaT} (at −20 mV: control: −0.85 ± 0.17 pA/pF, endostatin: −0.71 ± 0.16 pA/pF, n=6, data not shown), indicating its role in the pathogenesis of cardiovascular diseases. Three types of α_1-subunit genes encode

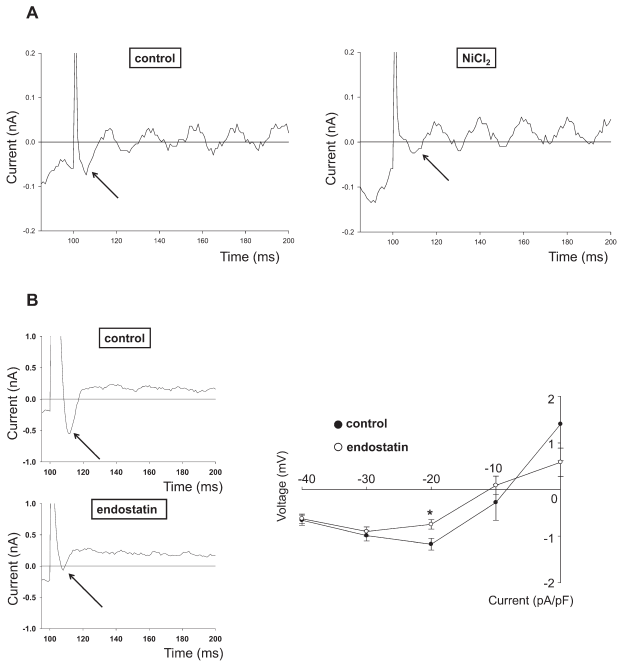

Fig. 2. Endostatin inhibited T-type Ca^{2+} channel current in isolated guinea pig ventricular myocytes. Representative T-type Ca^{2+} channel current (I_{CaT}) records (arrows) calculated by subtracting the current of a V_{HP-40} (holding potential: −40 mV) from the current of a V_{HP-90} (holding potential: −90 mV) at −20 mV in the same cell are shown (A, control: left, $NiCl_2$: right; B, control: upper left, endostatin: lower left). Endostatin (300 *n*g/m*l*) was pre-treated for 5 min. Current-Voltage relations for the peak currents of I_{CaT} (−40–0 mV) in the absence (closed symbols) or presence (open symbols) of endostatin are shown (right). The current density (pA/pF) was calculated by normalizing current amplitude by cellular membrane capacitance. Results were expressed as means ± S.E.M (n=11). * $P<0.05$ vs. control.

T-type Ca^{2+} channels: $Ca_v3.1$, 3.2 and 3.3. In mammals, $Ca_v3.1$ and 3.2 are known to be expressed in cardiac cells [15]. Zhang *et al*. reported that endostatin inhibited $Ca_v3.1$ and $Ca_v3.2$, but not $Ca_v3.3$ in human glioblastoma cell [20]. Therefore, endostatin might inhibit $Ca_v3.1$ and/or $Ca_v3.2$ in ventricular myocytes. The present study did not clarify how endostatin inhibits T-type Ca^{2+} channels. It was suggested in glioblastoma cells that endostatin inhibited T-type Ca^{2+} channels directly rather than via other pathways including G-protein and protein tyrosine kinase [20]. Further investigations are needed to clarify the inhibitory mechanisms of endostatin on T-type Ca^{2+} channels.

In conclusion, we for the first time demonstrated that endostatin can inhibit T-type Ca^{2+} channels in cardiac myocytes. It has been reported that Ca^{2+} signaling pathway activated through the T-type Ca^{2+} channels has an important role in the development of cardiac hypertrophy. Therefore, endostatin can be a new pharmaceutical target for the treatment of cardiac hypertrophy.

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