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Endocardium modulates myocardial contractile performance in isolated guinea pig papillary muscles[1]

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ABSTRACT Selective removal of endocardium by 1-s immersion of the muscle into 0.5% Triton X-100 resulted in a significant reduction of PT (peak isometric twitch tension) at 1.25 mmol·L^{-1} $[Ca^{2+}]_o$ over the stimulation frequency from 0.2 to 2.0 Hz (3.2 ± 0.2 *vs* 4.4± 0.4 mN/mm^2 at 1 Hz, $P<0.01$), while $+dT/dt_{max}$ was unaltered. Tension-$[Ca^{2+}]_o$ relation was shifted in accordance to $[Ca^{2+}]_o$, but with no significant change on PT at high $[Ca^{2+}]_o$ compared with endocardium-intact muscles. TPT (time to peak isometric tension) and) $RT_{\frac{1}{2}}$ (half isometric relaxation time) were typically shortened at all $[Ca^{2+}]_o$ or various stimulation frequencies (TPT: 203± 18 *vs* 265± 37 ms; $RT_{\frac{1}{2}}$: 77± 10 *vs* 108± 26 ms, at 1.25 mmol·L^{-1} and 1 Hz, $P<0.01$). Stimulation duration-threshold curve was slightly shifted to the left, yet no change in ERP (effective refractory period) was found. The data demonstrated that endocardium was an important modulator of myocardial contractile performance.

KEY WORDS endocardium; myocardial contraction; papillary muscles; calcium; electrophysiology

The essential role of endothelium in regulating vascular tone by releasing vasoactive substances has recently been appreciated[1,2]. Despite extensive knowledge about the role of vascular endothelium, the unique structural feature and physiological functions of endocardium had long been underestimated. Endocardium, ontogenetically akin to endothelium, consists of a monolayer of closely apposed endothelial cells covering the internal surface of the myocardium and shows a remarkable complexity of trabeculation which may substantially amplify its surface area. The development of endocardium precedes that of myocardium and, later still, of subsequent innervation and coronary vascularization[3]. Endocardium may modulate the contractile twitch behavior of myocardium in cats[4]. In the present study, we investigated and characterized the cardiotonic responses to selective removal of functional endocardium in isolated guinea pig papillary muscles.

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MATERIALS AND METHODS

Papillary muscle preparation Papillary muscles were isolated from the right ventricle of guinea pig and suspended horizontally in a specially designed miniature-bath (1.5 ml)[5] containing Krebs-Ringer solution bubbled with 95% O_2 + 5% CO_2, pH 7.35-7.45. The muscles of relatively cylindrical uniformity with cross-sectional area < 1.0 mm^2 were selected in order to ensure adequate oxygenation of all the muscle fibers. The nontendinous end of the papillary muscle was fixed to a spring-loaded clamp, while the tendinous end was directly attached to an isometric tension transducer (UC2, Gould Statham, USA). The preparations were perfused at a rate of 4 ml·min^{-1} and stimulated at a voltage about 10% above threshold by rectangular pulses of 5-ms duration through 2 platinum electrodes arranged in parallel with the entire muscle. The Krebs-Ringer solution contained NaCl 118, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 4.5, $CaCl_2 \cdot 2H_2O$ 1.25, 2.5, 5.0, or 7.5 mmol·L^{-1}.

Experimental protocol Preparations were allowed to stabilize in a solution containing $[Ca^{2+}]_o$ 1.25 mmol·L^{-1} at a resting tension of 50-100 mg for 2 h at 30℃ before starting the experiment. The muscle tension was measured by increasing the muscle length with a micrometer in 0.1-mm steps from L_o (muscle length at which minimal tension was developed)

to L_{max} (muscle length at which active force development was maximal), at which all experiments were performed. The isometric tension transducer attached to the tendinous end enabled measurements of resting tension (RT), peak isometric developed tension (PT), peak rate of tension rise and tension fall ($\pm dT/dt_{max}$), time to PT (TPT), and time to half-isometric relaxation ($RT_{\frac{1}{2}}$). The variables were simultaneously recorded by a polygraph (RM-6000, Nihon Kohden). Excitability of muscles was evaluated by means of stimulation duration-threshold relation and the ERP (effective refractory period) by the method described previously[6]. Force and velocity measurements were normalized by muscle cross sectional area (MCSA), which was calculated at the end of each experiment by dividing the lightly blotted wet weight of the muscle by its length at L_{max} (a cylindrical shape and a specific gravity of 1.0 were assumed). The effects of endocardium on inotropic responses were studied by performing paired experiments in muscles with or without intact endocardium, ie, only one intervention was studied in any single preparation. In some preparations, the influence of endocardium on the relationship between $[Ca^{2+}]_o$ and contractile performance was investigated in Krebs-Ringer solutions containing 1.25, 2.5, 5.0, or 7.5 mmol·L^{-1}. The contractile properties were also observed over a wide range of stimulation frequency from 0.2 to 2.0 Hz before and after selective removal of functional endocardium.

Selective removal of functional endocardium A modification of the method of Brutsaert *et al*[4] was used. Endocardial endothelium was selectively damaged in some preparations at the end of stabilization period by an 1-s immersion of the muscle into 0.5% Triton X-100 (Sigma Co) dissolved in oxygenated Krebs-Ringer solution at 30℃ and immediately followed by a rapid abundant wash with warm Ringer solution. A 1-s exposure time is only a minimal fraction of the time needed for detergent treatment to damage cellular membranes. Subjacent myocardium was undamaged as assessed morphologically by scanning and transmission electron microscopies and functionally by an unaltered maximal isometric tension at high $[Ca^{2+}]_o$ in our experiment. In contrast to untreated muscles, the muscles subjected to Triton X-100 manipulation of the endocardium was consistently stained with Evans blue following superfusion of the muscle surface with the dye and rapid wash with Krebs-Ringer solution. Evans blue is a highly charged compound that stains the muscle surface wherever there is loss of endocardium. Hence, following the experiments, this simple procedure provided an elegant check for irreversibly increased permeability and desquamation of the endocardial surface.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and compared with t test.

RESULTS

Effects of selective removal of endocardium Tab 1 showed the baseline characteristics of the muscle preparations used for the mechanical studies and the effects of selective removal of endocardium on the configuration of twitch contraction at 1 Hz, 30℃, and $[Ca^{2+}]_o$ 1.25 mmol·L^{-1}. In all the Triton X-100 treated muscles, an irreversible decrease occurred in peak isometric tension, while velocity of the tension development remained unchanged. No significant alterations in the passive length-tension properties of the muscles were observed in spite of a rare and slightly transient rise in resting tension in a few muscles. Meanwhile, endocardial removal resulted in a significant reduction in twitch duration and the onset of isometric relaxation occurred sooner as assessed by a decline in $RT_{\frac{1}{2}}$. Similarly, TPT and $T-(-dT/dt_{max})$ were typically shortened. However, the magnitude of reduction in peak twitch tension development or in twitch duration showed no significant correlation with MSCA. Once the phenomenon was established, the characteristic contractions remained stable for the entire course of the experiments and was not affected by slightly prolonging the duration of

Tab 1. Baseline characteristics of papillary muscles and effects of selective removal of endocardium on isometric twitch contraction at 1 Hz, 30℃, and $[Ca^{2+}]_o$ 1.25 mmol·L^{-1}. n= 6–7, $\bar{x} \pm s$. $^*P > 0.05$, $^{***}P < 0.01$ *vs* intact endocardium.

	Intact endocardium	Damaged–endocardium
L_{max}, mm	3.3± 0.8	3.1± 0.7
MSCA, mm^2	0.46± 0.18	0.41± 0.11
Threshold, V	2.3± 0.5	2.3± 0.4
RT, mN/mm^2	2.4± 0.5	2.8± 0.8
PT, mN/mm^2	4.4± 0.4	3.2± 0.2***
$+dT/dt_{max}$, $mN/mm^2/s$	22± 5	21± 5
$-dT/dt_{max}$, $mN/mm^2/s$	16± 3	18± 5
TPT, ms	265± 37	203± 18***
$RT_{\frac{1}{2}}$, ms	108± 26	77± 10***
T–(+dT/dt_{max}), ms	107± 14	96± 10*
T–(–dT/dt_{max}), ms	114± 13	77± 13***

immersion in 0.5% Triton X–100 to 2–s. Interestingly, the marked shortening of the contraction was not accompanied by changes in the velocity of tension development or peak contractile performance at high $[Ca^{2+}]_o$, further indicating that 1–s immersion in Triton X–100 caused no damage to the underlying myocardium.

The threshold voltage for the stimulation of the muscles was unaltered by rectangular pulses of 5–ms duration in endocardium–damaged preparations, but the threshold was significantly declined at 0.5 ms (Fig 1). The ERP was also examined in both endocardium–intact and –damaged muscles, but no change was seen (ERP: 388± 18 *vs* 387± 16 ms, $P > 0.05$).

Effects of endocardium on $[Ca^{2+}]_o$– and frequency–tension relations An increase in $[Ca^{2+}]_o$

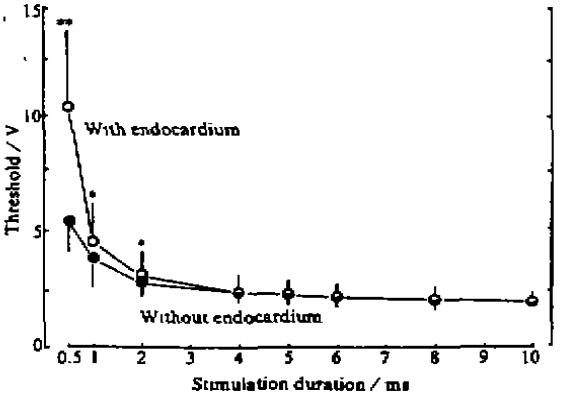

Fig 1. Stimulation duration–threshold voltage relationship in the paced (1 Hz) papillary muscles with or without intact endocardium. n= 7, $\bar{x} \pm s$. $^*P > 0.05$, $^{**}P < 0.05$ *vs* **without endocardium.**

resulted in a corresponding rise in PT in both endocardium–intact and –damaged muscles, while $RT_{\frac{1}{2}}$ and twitch duration were reduced, particularly in endocardium–intact muscle preparations (Fig 2). In those without intact endocardium, however, the $[Ca^{2+}]_o$–peak tension relationship was

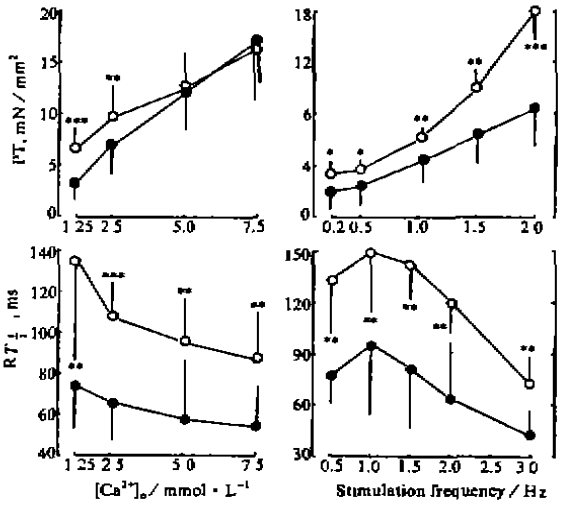

Fig 2. Effects of endocardium (○) on PT– and $RT_{\frac{1}{2}}$ – $[Ca^{2+}]_o$ and frequency relations, PT– and $RT_{\frac{1}{2}}$ – frequency relations. n= 5, $\bar{x} \pm s$. $^*P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* **without endocardium (●).**

asymmetrically shifted rightwards and downwards in accordance to the direction of increasing $[Ca_{2+}]_o$. Parameters of peak performance were significantly lowered after stripping of endocardium than those with intact one at $[Ca^{2+}]_o$ 1.25 mmol· L^{-1}. However, at $[Ca^{2+}]_o$ 5.0 mmol· L^{-1} or higher, they were similar in both preparations. $RT_{\frac{1}{2}}$ were significantly lowered after selective endocardial removal at all $[Ca^{2+}]_o$ studied as compared to those with intact endocardium.

An increase in stimulation frequency resulted in a corresponding rise in contractile performance in all preparations (Fig 2). The relationship between frequency and peak tension development in endocardium-damaged muscles was roughly in parallel with that of endocardium-intact ones. Damaging the endocardial surface led to a significant reduction of peak tension at 1, 1.5, and 2.0Hz. $RT_{\frac{1}{2}}$ was elevated initially, and then lowered with gradual rise in frequency in endocardium-intact muscles, while in endocardium- damaged preparations, $RT_{\frac{1}{2}}$ was shifted downwards significantly *vs* endocardium-intact muscles.

DISCUSSION

The present work proved that selective removal of functional endocardium resulted in a characteristic abbreviation of cardiac muscle twitch with concomitant reduction of peak isometric tension in isolated guinea pig papillary muscles. Our results were consistent with the studies in cats by Brutsaert *et al*[4,12]. *Since* $RT_{\frac{1}{2}}$ was typically shortened in all $[Ca^{2+}]_o$, while $+dT/dt_{max}$ was unaltered and no significant change on PT was found at $[Ca^{2+}]_o > 5.0$ mmol· L^{-1}, we proposed that the modulation of endocardium was more closely associated with relaxation of cardiac muscles and the reduction in PT occurred possibly as a result of the early onset of relaxation and consequently abbreviation of contraction cycle. Alternatively, the experimental results allowed us to postulate that the presence of an intact endocardium resulted in prolongation of the isometric twitch with a concomitant increase in peak twitch performance. This pattern of response contrasted to those observed in many other positive inotropic conditions where a marked increase in tension development was accompanied by a more or less abbreviated, rather than prolonged, contraction cycle. However, a response pattern similar to that could be observed in myocardial adaptation to the increase of initial muscle length and volume overloading through Frank-Starling Law and myocardial hypertrophy, respectively[7-9]. To what extent these mechanisms were linked or acted in concert to affect the cardiac performance remains unclear.

The mechanisms responsible for the modulation were only speculative at present. The endocardium might act as a physicochemical barrier to influence the transendothelial gradient for various ions[10], but evidences in favor of this were yet limited. A more attractive hypothesis is that the endocardial cells could release humoral substances locally: myocardium contracting factor (endocardin) and myocardium relaxing factor[11,12] which, in turn, affected the underlying myocardium, in a manner analogous to the role of vascular endothelium in regulating the vascular smooth muscle tone. Further studies on the underlying mechanism of the modulation by endocardium are currently in progress in our laboratory.

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心内膜调控离体豚鼠乳头状肌收缩反应

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摘要 选择性去除心内膜使离体豚鼠乳头状肌 PT 降低(3.2± 0.2 *vs* 4.4± 0.4 mN / mm^2), 其不受刺激频率的影响且± dT/dt_{max} 没有相应改变. 增加外钙浓度该作用减弱. 同时收缩反应时程缩短、舒张反应提前出现($RT_{\frac{1}{2}}$: 77± 10 *vs* 108± 26 ms), 刺激时程– 阈电压曲线轻度左移. 结果表明心内膜对心肌收缩反应具有特征性的调节作用.

关键词 心内膜; 心肌收缩; 乳头状肌; 钙; 电生理学

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Effects of rhynchophylline on motor activity of mice and serotonin and dopamine in rat brain[1]

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ABSTRACT Rhynchophylline (Rhy) reduced the spontaneous motor activity and enhanced the sedative and hypnotic effects of sodium pentobarbital in mice. The effects of Rhy on serotonin (5–HT) and dopamine (DA) concetrations in rat brain, and the release of 5–HT and DA from the regional brain slices were studied by a fluorescence detector. Rhy increased the 5–HT content in the hypothalamus and cortex, but reduced the DA concentrations in the cortex, amygdala, and spinal cord. Rhy promoted the release of endogenous DA from 4 brain regions. The release of 5–HT was increased in 2 brain regions and decreased in hypothalamus slice. However, Rhy inhibited the release of both 5–HT and DA evoked by high potassium.

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KEY WORDS rhynchophylline; motor activity; hypnotics and sedatives; serotonin; dopamine; central nervous system