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ENDOTHELIN IS A POSITIVE INOTROPIC AGENT IN HUMAN AND RAT HEART IN VITRO

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We have investigated the response to endothelin of isolated atrial and ventricular trabeculae from failing human hearts obtained at transplant. Results indicate that endothelin exerts a significant positive inotropic effect on human atrial and ventricular tissue, with increases in developed tension of 74.6± 14.1% (±SEM) and 9.9± 4.0%, respectively. Further studies on rat cardiac muscle demonstrate that the greater inotropic effect on atrial than ventricular muscle is also exhibited by the rat heart in vitro, with 39.9± 10.7% and 17.1± 5.9% increases in developed tension for atria and papillary muscle, respectively. Studies in rat atria also provide no evidence for an effect of endothelin on the frequency of spontaneous contractions. These results suggest that the potential exists for regulation of cardiac function in humans and rats by endothelial-derived factors such as endothelin, possibly via augmentation of atrial systole.

A regulatory role of the endothelium in mediating myocardial contractility has recently been investigated by Brutsaert and co-workers, who showed that the contractions produced by isolated cat papillary muscles could be depressed by removal of the endocardium (1). These observations suggest that the endocardium may be capable of exerting a modulatory influence on cardiac muscle contraction. Capillary endothelial cells, which come into close contact with myocardial cells (2), may provide additional sites for modulation of cardiac muscle function by the endothelium. We have investigated the question of endothelial modulation of human cardiac muscle, including the relative effects on atrial and ventricular tissue, using the recently characterized endothelial derived vasoconstrictor, endothelin (ET) (3). A recent report by Ishikawa and co-workers demonstrated that ET has a positive inotropic effect on isolated guinea

ABBREVIATIONS

ET: Endothelin; RT:Resting Tension; DT: Developed Tension; TPT: Time to Peak Tension; $T_{1/2}R$: Time to Half Relaxation; SR: Sarcoplasmic Reticulum.

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pig atria (4). We report here the results of our studies on human myocardium from heart failure patients and on cardiac muscle from a population of rats. We have studied the amplitude and timecourse of isometric tension development before and after the administration of ET in paced preparations of atrium and ventricle, as well as the influence of the peptide on the frequency of contraction in spontaneously beating rat atria. Our results indicate that ET exerts a significant positive inotropic effect on human cardiac muscle in vitro and that this effect is significantly greater on atrial than ventricular tissue. A follow-up study in rats indicates that rat atria and ventricular preparations in vitro are characterized by qualitatively similar positive inotropic responses to ET as human myocardium. Our results provide no evidence for an effect of ET on the frequency of spontaneous contractions in rat atria.

Methods

Atrial and ventricular trabecular muscles were obtained from seven heart failure patients undergoing cardiac transplantation at the Cleveland Clinic Foundation (5 men and 2 women, age range 16 - 52 years). Rat ventricular papillary muscles and isolated atria were obtained from male Sprague Dawley rats 10-12 weeks of age. Muscles were suspended in continually oxygenated Krebs-Henseleit buffer at 29° C between a force transducer and a stationary hook. Stimulation of 20% above threshold voltage, 5 ms duration and 0.2 Hz was delivered through parallel platinum electrodes throughout the experiment in all cases except for the spontaneously contracting rat atria. Four isometric contractile parameters were recorded for each muscle. These included the resting tension (RT; tension produced by the muscle in an unstimulated state as a function of its length), the developed tension (DT; active tension produced by the muscle when stimulated), the time to peak tension (TPT; time from the beginning of the contraction to the development of peak tension) and the time to half relaxation ($T_{1/2}R$; time from peak tension to half relaxation). In addition, the number of contractions per minute was recorded for the spontaneously contracting rat atria. Muscle length was adjusted to the point where maximal isometric tension was produced (L_{max}) and a dose of 50nM ET (Peninsula Laboratories, Belmont, CA) was added directly to the bath. We confirmed in dose-response curves that 50 nM ET caused a maximal inotropic response. The response of the muscle was recorded for 30 minutes after the peptide had been added to the bath. Length, width and weight of all preparations were determined and tension data were normalized for cross-sectional area of the human trabecular and rat papillary muscles. T-tests were used in order to determine whether a given contractile response to ET was significantly different from no response. Significant differences between the responses to ET of atrial and ventricular muscle from the same species were determined using unpaired t-tests. Results are reported as significantly different if $p < .05$. All results are reported as mean ± SEM.

Results

Baseline contractile data for atrial and ventricular trabeculae from human hearts are summarized in Table 1. Values are comparable to or greater than those reported by other investigators in similar studies (5,6). In response to the administration of 50 nM ET, human trabeculae exhibited a response which was slow in onset (taking 15-20 minutes to reach the maximum response), long lasting, and difficult to wash out. The percent change in each contractile parameter in response to ET is presented in Figure 1. Results are expressed as percent change since one of the main determining factors for altering cardiac performance is the ability of atrial or ventricular muscle to increase its

Table I. Baseline Contractile Response of Human and Rat Muscle at L_{max} (Mean ± SEM)

	Resting Tension (g/mm^2)	Developed Tension (g/mm^2)	Time To Peak Tension (ms)	Time to 1/2 Relaxation (ms)
Human atrial muscle (n=9)	0.70±0.10	0.30±0.10	184.40±7.00	141.10±10.70
Human ventricular muscle (n=9)	1.10±0.30	2.00±0.60	503.90±50.10	372.20±45.50
Rat atrial muscle (n=9)	0.93±0.04*	1.51±0.11*	65.56±2.82	47.78±2.06
Rat ventricular muscle (n=9)	1.51±0.22	3.44±1.01	131.67±5.59	117.78±11.0

*Units for rat atria are g rather than g/mm^2.

force of contraction relative to its own baseline. Both atrial and ventricular muscles from human hearts showed a significant increase in DT ($p<.001$ and $p<.05$ respectively, compared to baseline; $p<.01$ for atria compared to ventricle; Figure 1). In response to ET, ventricular trabeculae from human heart also showed a significant decrease in resting tension ($p<.05$).

Baseline contractile data for rat atria and rat ventricular papillary muscles are summarized in Table 1. Values are comparable to or greater than those reported by others (7, 8). Response to 50 nM ET in rat cardiac muscle was also slow in onset (taking 10-15 minutes to reach a maximum response), long lasting, and difficult to wash out. The percent change in each contractile parameter in response to ET is summarized in Figure 1. Atrial muscle from the rat demonstrated a significant decrease in RT ($p<.01$) and a significant increase in DT ($p<.01$ compared to baseline; $p<.05$ compared to rat ventricle); ventricular muscle showed a decrease in RT ($p<.01$), an increase in DT

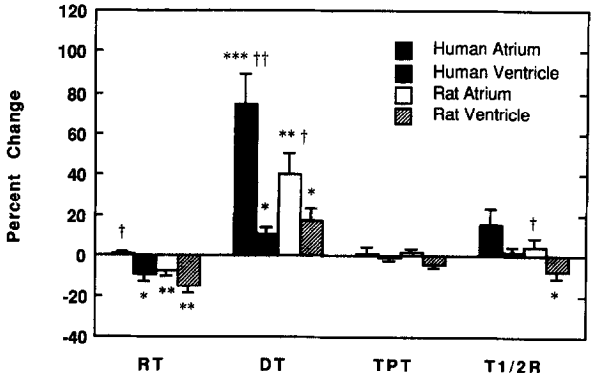

Figure 1: Response of human and rat tissues to 50 nM endothelin. Values indicated are mean ± SEM. RT: Resting Tension, DT: Developed Tension, TPT: Time to Peak Tension, $T_{1/2}R$: Time to Half Relaxation. *$p<.05$, **$p<.01$, ***$p<.001$ that response is significant. >$p<.05$ and >>$p<.01$ that response in atrial tissue is significantly different from response in ventricular tissue of the same species. (n = 9 for each of the four groups).

($p<.05$), and a decrease in $T_{1/2}R$ ($p<.05$). The effect of ET on $T_{1/2}R$ was also significantly different between atrial muscle (increased $T_{1/2}R$), and ventricular muscle (decreased $T_{1/2}R$) ($p<.05$).

In spontaneously contracting rat right atria ($n = 6$), the average number of contractions per minute at L_{max} was 174.0 ± 12.5 and this frequency did not change significantly (171.0 ± 14.3) after the addition of 50 nM ET. The positive inotropic effect of ET could also be demonstrated in these spontaneously contracting atria.

Discussion

Several studies have suggested that the endothelium itself, or an endothelial-derived factor, may have the potential to influence force generation by the myocardium (1,4). The primary purpose of the current study was to determine whether the potential for modulation by an endothelial derived compound also exists in the human heart. Data presented here indicate that ET can exert a positive inotropic effect on human cardiac muscle strips in vitro but that the effect is much greater in atrial than ventricular muscle. The action of ET on human myocardium is characterized by augmented contractile force with no significant changes in the timing parameters of isometric contraction, similar to the observations of Ishikawa et al. on the guinea pig atrium (4). Studies using rat atria also revealed a marked inotropic effect of ET. A smaller but significant response was also observed in rat ventricle.

The mechanism of action of ET on myocardial tissue remains to be established. Increased force development in response to ET suggests that increased intracellular calcium is made available to activate the contractile proteins. This mechanism of action is supported by studies in vascular smooth muscle, where the free cytosolic calcium concentration is increased following ET administration (9). The effect of ET has also been shown to be antagonized by slow calcium channel blockers in vascular smooth muscle (3) and heart (4). Unlike agents such as isoproterenol, which accelerate uptake of calcium into the sarcoplasmic reticulum (SR) and also significantly shorten TPT and $T_{1/2}R$ (10), ET did not affect the timing parameters of contraction in three out of four tissues studied. Even in rat papillary muscle, the 8% decrease in $T_{1/2}R$ that was observed in response to ET stimulation is much smaller than the 30-40% decrease in $T_{1/2}R$ that we have previously measured in response to isoproterenol. These results therefore suggest that acceleration of calcium uptake by the SR is not a major mechanism of action of ET in the myocardium.

The significant decrease in resting tension which accompanies ET administration in human ventricle and rat atrium and ventricle suggests that, in the presence of ET, the amount of cytosolic calcium present between contractions is significantly less than in the absence of ET. This indicates that ET may reduce cytosolic calcium levels below baseline or else decrease the calcium sensitivity of regulatory calcium binding proteins, such as troponin C.

Since endothelial-derived contracting factors have been reported to be released in response to stretch (11) and anoxia (12), both intrinsic to many cardiovascular disease states, it might be hypothesized that the contractility of the diseased myocardium is affected by the release of factors such as ET from the endocardium or from the capillary endothelial cells. Relevant to our demonstration of a greater effect of ET on atrial tissue, particularly in human heart, it is well established that augmented atrial systole can increase cardiac output by increasing diastolic filling of the ventricle (13,14).

In conclusion, our results demonstrate that both human and rat heart are capable of responding to inotropic modulation by at least one endothelial-derived compound. It has not been determined whether the observed response to ET typifies human heart in general or only the failing myocardium. The physiological significance of the ability of the heart to respond to endothelial derived factors remains to be established, but the results of this study raise the possibility that endocardial cells and/or capillary endothelial cells may contribute to the regulation of cardiac function.

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