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The rate of force generation by the myocardium is not influenced by afterload

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Abstract

The influence of afterload on the rate of force generation by the myocardium was investigated using two types of preparations: the *in situ* dog heart (dP/dt) and isolated papillary muscle of rats (dT/dt). Thirteen anesthetized, mechanically ventilated and thoracotomized dogs were submitted to pharmacological autonomic blockade (3.0 mg/kg oxprenolol plus 0.5 mg/kg atropine). A reservoir connected to the left atrium permitted the control of left ventricular end-diastolic pressure (LVEDP). A mechanical constriction of the descending thoracic aorta allowed to increase the systolic pressure in two steps of 20 mmHg (conditions H_1 and H_2) above control values (condition C). After arterial pressure elevations (systolic pressure C: 119 ± 8.1; H_1: 142 ± 7.9; H_2: 166 ± 7.7 mmHg; $P<0.01$), there were no significant differences in heart rate (C: 125 ± 13.9; H_1: 125 ± 13.5; H_2: 123 ± 14.1 bpm; $P>0.05$) or LVEDP (C: 6.2 ± 2.48; H_1: 6.3 ± 2.43; H_2: 6.1 ± 2.51 mmHg; $P>0.05$). The values of dP/dt did not change after each elevation of arterial pressure (C: 3,068 ± 1,057; H_1: 3,112 ± 996; H_2: 3,086 ± 980 mmHg/s; $P>0.05$). In isolated rat papillary muscle, an afterload corresponding to 50% and 75% of the maximal developed tension did not alter the values of the maximum rate of tension development (100%: 78 ± 13; 75%: 80 ± 13; 50%: 79 ± 11 g mm^{-2} s^{-1}, $P>0.05$). The results show that the rise in afterload *per se* does not cause changes in dP/dt or dT/dt.

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Introduction

The rate of left ventricular pressure development (dP/dt) has been widely used to evaluate myocardium contractility. Several studies (1-7) have suggested that dP/dt values depend on physiological factors altering cardiac performance other than inotropism, such as heart rate (HR) and preload.

Concerning the influence of afterload on dP/dt, the available data (1-3,5,7-15) point to a very controversial situation. From a linear point of view, it would be natural to expect that the maximal dP/dt value is not changed by the aortic diastolic pressure rise since its maximum positive value occurs before aor-

tic valve opening takes place (6,8,16). However, the results of the studies which evaluated the influence of afterload on dP/dt are discrepant. Some investigators (3,8-12) have reported that the rise in arterial pressure (AP) did not interfere with the dP/dt values, whereas others (1,2,5,7,13-15) reported a direct relationship between AP rise and dP/dt. The divergent results may be due to different influences of other variables which do not present the same behavior in these different studies, such as preload and coronary perfusion pressure or coronary flow.

The purpose of the present study was to re-examine the effects of afterload variations on the rate of force generation by the myocardium, trying to eliminate the preload influence. We studied the effect of afterload elevations on dP/dt in the *in situ* dog heart and on dT/dt in isolated papillary muscle preparations. In the*in situ* dog heart preparation, the AP was elevated while the left ventricular end-diastolic pressure (LVEDP) was kept constant. In the isolated muscle preparation a constant preload and myocardium nutrition allowed by diffusion eliminated the Frank-Starling mechanism and also the influences of coronary perfusion on dT/dt.

Material and Methods

In situ dog heart

Thirteen mongrel dogs weighing 15 to 35 kg, anesthetized with 2 mg/kg meperidine, *im*, and 60 mg/kg chloralose and 600 mg/kg urethan, *iv*, and mechanically ventilated were submitted to midline thoracotomy. The thoracic aorta was repaired with cardiac tape below the emergence of the left subclavian artery. The pericardium was opened from the apex of the heart to the emergence of the pulmonary artery and used to support the heart as a pericardial cradle permitting a suitable exposure of the left atrium (LA) appendage. A catheter (4 cm long, 1.4 mm internal diameter) was introduced into the left ventricular cavity through the apex of the heart and the tip of another catheter (100 cm long, 1.8 mm internal diameter) was placed in the ascending aorta from the left femoral artery and the distal extremities of both catheters were connected to pressure transducers (Statham P23-ID). Left ventricular pressure, dP/dt and aortic pressure were obtained with a model 1205 pressure amplifier connected to an Electronics for Medicine VR-12 physiologic recorder. The left ventricular pressure was amplified as low and high gain records in order to analyze systolic and diastolic pressures. The dP/dt ratio was obtained utilizing the differentiating circuit of the pressure amplifier (time constant: 0.1 s; linear response until 200 Hz). These variables and a bipolar lead of the electrocardiogram were continuously monitored.

The intravenous administration of 0.5 mg/kg atropine and 3.0 mg/kg oxprenolol blocked parasympathetic and ß-adrenergic activities. The ß-adrenergic blockade obtained with oxprenolol was also sufficient to abolish the inotropic and chronotropic effects of isoproterenol (1 µg/kg, *iv*).

The LVEDP was maintained constant during the rise in AP by using a circuit attached to the LA. The circuit included a reservoir with a limiting level system draining the blood of the LA appendage to another reservoir and a roller pump which pumps the blood to the jugular veins of the animal. When the ventricular filling pressure began to rise, the level of the liquid in the reservoir rose, allowing the blood to drain from the limiting level system to the second reservoir, from which it was then pumped to the dog jugular veins. Thus, the left ventricular filling pressure was kept at the initial value throughout the experiment.

After a 15-min stabilization period, the height of the reservoir was fixed to maintain the basal LVEDP level constant. When the preparation was stable, the variables were recorded under control (C) conditions after

10 s of expiratory apnea.

By means of the cardiac tape, the aorta was constricted until the systolic pressure was increased by approximately 20 mmHg above control levels and arterial hypertension was sustained for 10 min in order to obtain the hypertension 1 condition (H_1). At the end of this period, the variables were recorded. The aortic constriction was released and the preparation was allowed to stabilize for 10 min. Thereafter, these procedures were repeated to increase arterial pressure by approximately 40 mmHg above control conditions and, after 10 min, the variables were again recorded as hypertension 2 condition (H_2). The limiting system did not allow variations in the ventricular filling pressure during AP changes.

The following variables were analyzed: HR, left ventricular systolic pressure (LVSP), LVEDP, diastolic aortic pressure (DAP) and dP/dt.

Isolated papillary muscle

Ten male Wistar rats weighing 204 to 232 g were killed by decapitation. The hearts were quickly removed and placed in oxygenated Krebs-Henseleit solution at 28ºC. Trabecular carneae or papillary muscles were dissected from the left ventricle, mounted between two spring clips, and placed vertically in a chamber containing Krebs-Henseleit solution at 28ºC and gassed with 95% O_2 and 5% CO_2. The composition of the Krebs-Henseleit solution was as follows: 118.5 mM NaCl, 4.69 mM KCl, 2.52 mM $CaCl_2$, 1.16 mM $MgSO_4$,1.18 mM KH_2PO_4, 5.50 mM glucose, and 25.88 mM $NaHCO_3$. The lower spring clip was attached to a Kyowa model 120T-20B force transducer by a thin steel wire (1/15,000 inch), which passed through a mercury seal at the bottom of the chamber. The upper spring clip was connected by a thin steel wire to a rigid lever arm above which a micrometer stop was mounted for the adjustment of muscle length. Preparations were stimulated 12 times/min with 5-ms square wave pulses through parallel platinum electrodes, at voltages 10% higher than the minimum required to produce a maximal mechanical response.

After a 60-min period during which the preparations were allowed to shorten while carrying light loads, muscles were loaded to contract isometrically and stretched to the apex of their length-tension curves (L_{max}).

After a 5-min period during which preparations performed afterloaded isotonic contractions, muscles were again placed under isometric conditions, and the apex of the length-tension curve (L_{max}) was again carefully determined. A 15-min period of stable isometric contraction was imposed prior to the experimental period and one isometric contraction was then recorded. Thereafter, the muscle was allowed to perform afterloaded isotonic contraction in which the developed tension was constant during muscle shortening. The level of developed tension was determined so that the active tension could reach 75% and, in the next step, 50% of the developed tension obtained in the isometric contraction. Resting tension was not changed, since the muscle was maintained at L_{max} during the afterload variations. Under the present afterload conditions all the contractions were recorded after muscle stabilization.

The following parameters of the isometric contractions were measured: developed tension (DT, g/mm^2), resting tension (RT, g/mm^2), time to peak tension (TPT, ms) and maximum rate of tension development (dT/dt, g mm^{-2} s^{-1}). For afterloaded contractions, DT, RT and dT/dt were assessed.

At the end of each experiment, the muscle length at L_{max} was measured and the muscle between the two clips was blotted dry and weighed. Cross-sectional areas were calculated from the muscle weight and length by assuming cylindrical uniformity and a specific gravity of 1.0. All force data were normalized for the muscle cross-sectional area.

Statistical analysis

Data are reported as mean ± SD. The comparisons of HR, LVSP, DAP, LVEDP and dP/dt values obtained under the C, H_1 and H_2 conditions, as well as dT/dt values verified in the different afterloads, were made by repeated measurement analysis (multivariate analysis - mean profile). Differences were considered to be significant when $P<0.05$ (17).

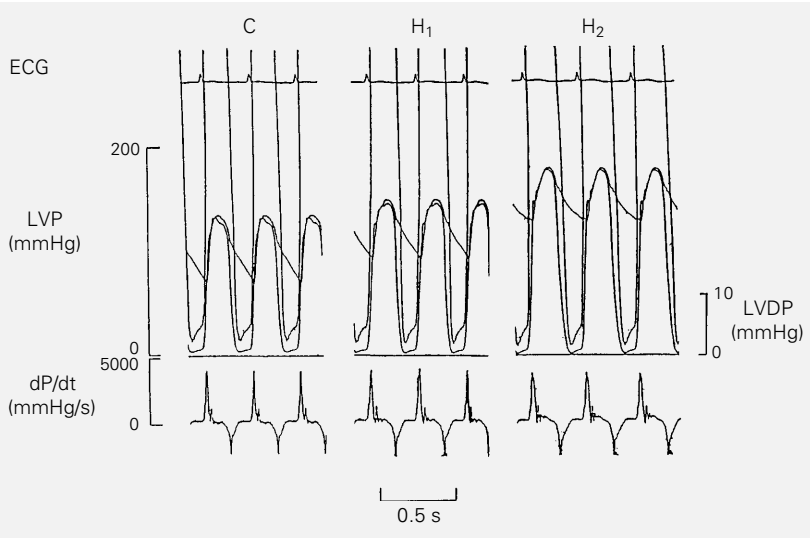

Figure 1 - Record from one *in situ* dog heart experiment. Effect of sustained elevation (10 min) of aortic pressure on dP/dt with constant left ventricular end-diastolic pressure. C = Control, H_1 = hypertension 1, H_2 = hypertension 2, ECG = electrocardiogram, LVP = left ventricular pressure, LVDP (left ventricular diastolic pressure) = LVP at higher gain to show diastolic part (systole off scale), dP/dt = rate of rise of the left ventricular pressure.

Table 1 - Effects of arterial pressure increases on cardiac parameters of dogs.

Data are reported as mean ± SD (N = 13). HR = Heart rate; LVSP = left ventricular systolic pressure; DAP = diastolic aortic pressure; LVEDP = left ventricular end-diastolic pressure; dP/dt = rate of rise of the left ventricular pressure; C = control; H_1 = hypertension 1 condition; H_2 = hypertension 2 condition. *P<0.01 *vs* C; $^{+}$P<0.01 *vs* H_1 (repeated measurement analysis).

	HR (bpm)	LVSP (mmHg)	DAP (mmHg)	LVEDP (mmHg)	dP/dt (mmHg/s)
C	125 ± 13.9	119 ± 8.1	89 ± 11.6	6.2 ± 2.48	3,068 ± 1,057
H_1	125 ± 13.5	142 ± 7.9*	99 ± 9.5*	6.3 ± 2.43	3,112 ± 996
H_2	123 ± 14.1	166 ± 7.7*$^{+}$	120 ± 11.8*$^{+}$	6.1 ± 2.51	3,086 ± 980

Results

In situ dog heart preparation

Table 1 shows the data obtained under the C, H_1 and H_2 conditions. Statistical analysis revealed that LVSP (C: 119 ± 8.1; H_1: 142 ± 7.9; H_2: 166 ± 7.7 mmHg) and DAP (C: 89 ± 11.6; H_1: 99 ± 9.5; H_2: 120 ± 11.8 mmHg) were significantly different ($C< H_1<H_2$, $P<0.01$). There was no significant variation in HR (C: 125 ± 13.9; H_1: 125 ± 13.5; H_2: 123 ± 14.1 bpm), LVEDP (C: 6.2 ± 2.48; H_1: 6.3 ± 2.43; H_2: 6.1 ± 2.51 mmHg) or dP/dt (C: 3,068 ± 1,057; H_1: 3,112 ± 996; H_2: 3,086 ± 980 mmHg/s) during AP changes. Figure 1 shows the records obtained in one of the experiments.

Isolated papillary muscle

Table 2 shows the basal data obtained during the isometric contraction of the muscles at L_{max}. Table 3 shows the values of peak dT/dt under the different afterloaded conditions analyzed in the isolated muscle strips. Peak dT/dt was obtained at contractions with developed tension levels corresponding to 100% (dT/dt: 78 ± 13 g mm^{-2} s^{-1}), 75% (dT/dt: 80 ± 13 g mm^{-2} s^{-1}), and 50% (dT/dt: 79 ± 11 g mm^{-2} s^{-1}) of the developed tension verified in isometric contraction. No significant difference ($P>0.05$) was found in peak dT/dt values at the three different afterloads.

Discussion

Our results obtained with the isolated papillary muscle preparations support the view that the amount of developed force did not interfere with the intensity of the activation process. At present, two known factors are suggested as potentially affecting the rate of myocardial force generation when afterload changes: the Anrep effect (15,18-20) and the shortening deactivation (21-23). Until

recently, there was no consistent evidence that the Anrep effect could be demonstrated in muscle strips. A few years ago, Nichols et al. (20) convincingly demonstrated the occurrence of the Anrep effect in muscle strips specially instrumented in order to obtain "physiological" contraction. In isolated muscles studied in isometric and afterloaded contractions as those utilized by us, it was not possible for these authors to identify the influence of the Anrep effect on the contraction. On the other hand, contraction deactivation due to shortening has been described under various types of experimental conditions (21-24). It seems valid to assume that shortening deactivation did not occur in our study. Indeed, if shortening deactivation had occurred, peak dT/dt should have proportionally decreased in contractions studied under afterloaded conditions, i.e., 75% and 50% of isometric contractions. Recently, Konishi et al. (7) described a direct correlation between afterload and peak dT/dt in contractions analyzed in a range of force that was 14% to 70% of isometric contractions. It seems that the wide range of developed force could facilitate the occurrence of shortening deactivation. Moreover, such a small afterload should prevent the occurrence of the maximal dT/dt value since force development was interrupted early (8,9).

In the *in situ* canine heart preparation, the rise in AP also did not promote any increase in the dP/dt values. Since we maintained the left ventricular filling pressure constant during the rise in aortic pressure, we prevented the participation of the Frank-Starling mechanism in our experiments and eliminated a spurious factor of interference with the results that others have not provided (2,13).

According to present knowledge, other factors that may alter the dP/dt values after increasing AP are shortening deactivation (21-24), the Anrep effect (15,18,19) and variations in coronary flow (25) or coronary perfusion pressure (26). Previous studies from our laboratory have shown that large increases of developed force (27) and coronary perfusion (28) do affect left ventricular performance in the intact canine left ventricle. The influence of afterload is thought to be linked to the Anrep effect and, typically, occurs as a cyclic and transitory oscillation of cardiac performance (29). There is an initial impairment of contraction followed by improvement of ventricular performance until complete recovery. A reduction of the subendocardial coronary flow followed by restoration of myocardial perfusion seems to be implicated when the Anrep effect occurs in the intact heart (18,19). Complete cardiac influence of the Anrep effect is limited to a few minutes, and is certainly not present at the end of 10 min. It has been described that, in addition to myocardial ischemia, coronary perfusion pressure can affect cardiac mechanics interfering with intracellular calcium concentration (30,31) and sarcomere resting length (26,32). Abel and Reis (25) described an enhancement of cardiac performance following the increase of coronary

Table 2 - Isometric contraction data of rats.

Data are reported as mean ± SD (N = 10). CSA = Cross-sectional area; DT = developed tension; RT = resting tension; TPT = time to peak tension; dT/dt = maximum rate of tension development.

CSA	0.75 ± 0.11 mm^2
DT	8.21 ± 1.62 g/mm^2
RT	0.55 ± 0.19 g/mm^2
TPT	186 ± 14 ms
dT/dt	78 ± 13 g mm^{-2} s^{-1}

Table 3 - Effect of afterload changes on dT/dt.

Data are reported as mean ± SD (N = 10). DT = Developed tension; dT/dt = maximum rate of tension development (P>0.05 for dT/dt; repeated measurement analysis).

	Afterload (% of DT in isometric contraction)		
	50%	75%	100%
DT (g/mm^2)	4.0 ± 0.88	6.11 ± 2.5	8.21 ± 1.62
dT/dt (g mm^{-2} s^{-1})	79 ± 11	80 ± 13	78 ± 13

flow without changes in perfusion pressure. The mechanisms underlying this effect of flow on cardiac mechanics are unknown. Since in the *in situ* dog heart preparations arterial pressure has always been manipulated in order to increase AP, the cumulative effects of shortening deactivation, Anrep effect and coronary perfusion should enhance ventricular performance. In spite of the possible concurrence of these factors in heightening contraction, an elevation of peak dP/dt was not verified. It seems pertinent to conclude that, in the *in situ* dog heart, variations in arterial pressure within wide physiological ranges do not affect *per se* the maximal values of dP/dt. A particular exception is represented by those situations in which there is an expressive reduction in diastolic arterial pressure. Under such conditions, the values of dP/dt would not represent the maximum contractile performance of cardiac muscle, since premature shortening prevents the actual peak of dP/dt. Moreover, coronary flow should be impaired when diastolic arterial pressure is under the values of autoregulatory mechanism. This seems to be a fitting interpretation for the diverging results recently described by Konishi et al. (7) studying working rat heart preparations. These investigators utilized peak systolic pressures ranging from 66 to 97 mmHg and found that the rise in aortic pressure was accompanied by elevations of peak dP/dt. This seems to be an inconvenient pressure range to be studied.

In conclusion, the present results suggest that the increase in afterload does not cause changes in the dP/dt or dT/dt values.

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