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# The left ventricular contractility of the rat heart is modulated by changes in flow and $\alpha_1$-adrenoceptor stimulation

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## Abstract

Myocardial contractility depends on several mechanisms such as coronary perfusion pressure (CPP) and flow as well as on $\alpha_1$-adrenoceptor stimulation. Both effects occur during the sympathetic stimulation mediated by norepinephrine. Norepinephrine increases force development in the heart and produces vasoconstriction increasing arterial pressure and, in turn, CPP. The contribution of each of these factors to the increase in myocardial performance needs to be clarified. Thus, in the present study we used two protocols: in the first we measured mean arterial pressure, left ventricular pressure and rate of rise of left ventricular pressure development in anesthetized rats (N = 10) submitted to phenylephrine (PE) stimulation before and after propranolol plus atropine treatment. These observations showed that *in vivo* $\alpha_1$-adrenergic stimulation increases left ventricular-developed pressure ($P<0.05$) together with arterial blood pressure ($P<0.05$). In the second protocol, we measured left ventricular isovolumic systolic pressure (ISP) and CPP in Langendorff constant flow-perfused hearts. The hearts (N = 7) were perfused with increasing flow rates under control conditions and PE or PE + nitroprusside (NP). Both CPP and ISP increased ($P<0.01$) as a function of flow. CPP changes were not affected by drug treatment but ISP increased ($P<0.01$). The largest ISP increase was obtained with PE + NP treatment ($P<0.01$). The results suggest that both mechanisms, i.e., direct stimulation of myocardial $\alpha_1$-adrenoceptors and increased flow, increased cardiac performance acting simultaneously and synergistically.

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**Key words**
- Myocardial performance
- Coronary perfusion pressure
- Coronary flow
- Phenylephrine

## Introduction

Myocardial contractility depends on several mechanisms including the increase of coronary perfusion pressure (CPP) and flow as well as autonomic nervous system stimulation. Increases of coronary flow (1-3) and perfusion pressure (4-7) are reported to increase myocardial contractility. Also, increased sympathetic activity enhances contractility, activating $ß_1$ and $\alpha_1$-adrenoceptors. $ß_1$-adrenoceptor stimulation is known to increase heart rate and contractility acting on G-proteins and increasing intracellular cAMP concentration (8). Stimulation of $\alpha_1$-adrenoceptors increases force development in the myocardium acting on G-proteins and inducing phospholipase C activation (9-13). At the same time the action on vascular smooth muscle (VSM) produces peripheral

vasoconstriction that increases arterial pressure and, consequently, the coronary perfusion pressure and flow (3). All these effects may occur simultaneously during sympathetic stimulation produced by the effects of norepinephrine on the heart and on the VSM of the arterial systemic circulation.

However, it is not known whether direct action on the myocardium or the increase in coronary perfusion pressure and flow contributes more to the increase in myocardial contraction that occurs during $\alpha_1$-sympathetic stimulation. To elucidate this point, the effects of $\alpha_1$-adrenoceptor stimulation were studied in anesthetized rats and in rat hearts perfused with increasing flow rates.

## Material and Methods

Care and use of laboratory animals were according to NIH guidelines. All rats had free access to water and were fed with rat chow *ad libitum*.

Male Wistar rats (EPM strain) weighing 250 to 350 g (N = 17) were divided into two groups. In the first group (N = 10), the rats were anesthetized *ip* with urethane (1.8 g/kg) and the right carotid artery and jugular vein were cannulated. Urethane was supplemented (0.45 g/kg) when rats showed tachypnea plus movement of mystacial vibrissae. The anesthetized rats were allowed to breathe room air spontaneously. A PE50 cannula was introduced through the carotid artery into the left ventricle (LV). Left ventricular systolic pressure (LVSP) and its peak first time derivative ($dP/dt_{max}$) were measured using a pressure transducer (Gold P23XL) connected to an amplifier (MP100-FUNBEC) and recorded with a Nihon-Kohden (RM-6000) polygraph. Mean arterial pressure (MAP) was recorded at the femoral artery level and the ECG was recorded with a bioelectric amplifier (ME 100 FUNBEC) as carried out for people. A venous cannula in the jugular vein was used for drug infusion.

In the second group (N = 7), animals were injected with heparin (50 IU) and killed 10 min later by cervical dislocation. The heart was rapidly removed, transferred to a Langendorff apparatus and perfused through the aortic stump under a constant flow (10 ml/min) which was changed when necessary at a temperature of 31°C. The composition of the nutrient solution was: 120 mM NaCl, 5.4 mM KCl, 1.0 mM $MgCl_2$, 1.25 mM $CaCl_2$, 27 mM $NaHCO_3$, 2.0 mM $NaH_2PO_4$, 1.2 mM $Na_2SO_4$, and 11.0 mM glucose. The solution was previously filtered with a 0.8-µm Millipore filter and continuously gassed with 95% $O_2$ + 5% $CO_2$ mixture (pH = 7.3-7.4). The right atrium was excised and paced at 200 beats/min. The left atrium was then opened to introduce a soft distensible balloon mounted at the tip of a rigid plastic tube into the left ventricular cavity through the atrioventricular valve. To avoid liquid accumulation in the ventricular cavity, the ventricle was perforated with a puncture needle. The balloon was connected to a pressure transducer (Gould P23XL) and to a syringe via a Y piece, so that the diastolic pressure of the LV could be adjusted to predetermined values by injecting water into the balloon. Developed isovolumic systolic pressure (ISP) was measured with a chart recorder (FUNBEC RG300). CPP was also measured at the aortic cannula level. Measurements were initiated after a period of stabilization of 30 min with the heart driven at 200 beats/min.

### Experimental protocols

#### *Anesthetized rats*

After cannulation of the LV the effects of *iv* bolus injections of 10 ng phenylephrine (PE) on LVSP and its dP/dt were determined. To avoid the interference of regulatory autonomic reflexes the protocol was repeated before and after ß-adrenergic plus cholinergic blockade with propranolol (3 mg/

kg) plus atropine sulfate (1 mg/kg) treatment. Mean blood pressure was measured simultaneously.

#### *Langendorff-perfused hearts*

The basic protocol was performed beginning with a constant diastolic pressure of 4 to 7 mmHg by adjusting the volume of the balloon. During the experiments the volume of the balloon was kept constant, thus permitting the measurement of the diastolic and systolic pressure changes produced by each perfusion flow with nutrient solution alone or containing PE or nitroprusside (NP) + PE. First, under control conditions the flow was increased in progressive steps of 5 ml/min (5, 10, 15 and 20 ml/min). The same protocol was then repeated with hearts perfused with 0.5 µg/ml PE until systolic pressure stabilization, which was attained after 10 min. In the third protocol, the PE perfusion solution was supplemented with 100 mg/ml NP to induce coronary vasodilatation. This procedure was used to investigate if the effects of PE were changed by complete vasodilatation.

Phenylephrine, propranolol, atropine, urethane and nitroprusside were purchased from Sigma Chemical Co., St. Louis, MO.

#### Statistical analysis

Data are reported as means ± SEM. Repeated measures ANOVA was used to compare means among groups. When the results of ANOVA were significant, the Tukey test was used to compare means. Statistical significance was set at $P<0.05$.

## Results

#### *In vivo* experiments

In this protocol the effects of $\alpha_1$-adrenoceptor stimulation were studied in anesthetized rats before and after ß-adrenergic and cholinergic blockade. Table 1 shows that anesthetized rats increased their LVSP in response to phenylephrine administration, and changes in MAP were similar to those observed for LVSP, showing increases of 17 ± 2.39 mmHg. Phenylephrine produced a small nonsignificant increase of dP/dt. To avoid the influence of autonomic reflexes resulting from $\alpha_1$-adrenoceptor stimulation, propranolol plus atropine were administered to achieve a ß-adrenergic and cholinergic blockade. The protocol was repeated after 20 min and LVSP was reduced. PE administration produced an increase of LVSP which was not followed by dP/dt increments, while MAP increased 23.8 ± 2.4 mmHg.

#### *In vitro* experiments

Figure 1 shows the relationship between increases of flow and CPP and between flow and ISP. A significant positive linear correlation between flow and CPP was obtained ($R = 0.88$, $P<0.0001$) as expected, since in this preparation CCP is directly related to flow and vascular coronary resistance. A positive linear correlation was also observed between ISP and flow ($R = 0.51$, $P<0.001$), suggesting interdependence between these two variables (Figure 1).

To evaluate the dependence of the effects of $\alpha_1$-adrenergic stimulation during flow increases the preparations were perfused with

Table 1 - Effects of phenylephrine (PE) on left ventricular systolic pressure (LVSP) and rate of rise of pressure (dP/dt) in anesthetized rats before and after sympathetic and parasympathetic blockade with propranolol and atropine.

Results are reported as means ± SEM. *P<0.05 *vs* each respective control value (two-way ANOVA).

| | Before blockade | | After blockade | |
|---|---|---|---|---|
| | Control | PE | Control | PE |
| LVSP (mmHg) | 113 ± 7.44 | 126 ± 5.6* | 89.5 ± 5.66 | 107 ± 1.22* |
| dP/dt (mmHg/s) | 1635 ± 213 | 1657 ± 274 | 1370 ± 144 | 1428 ± 192 |

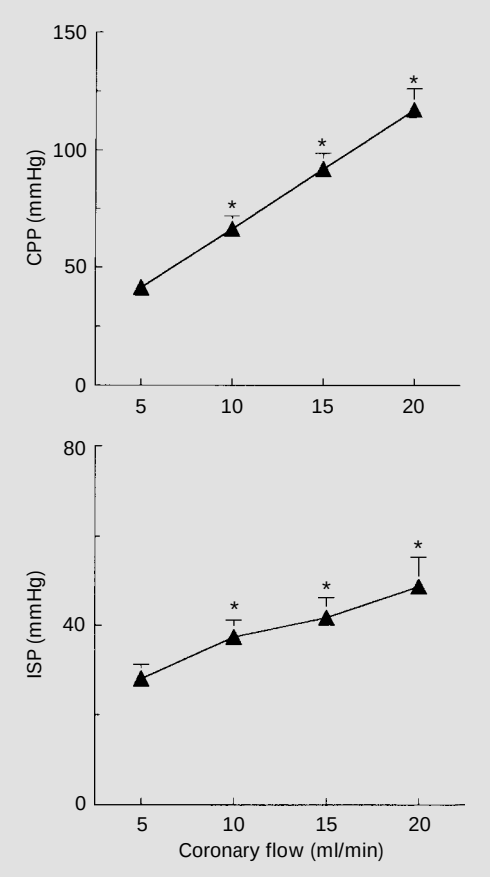


Figure 1 - Effects of increases of coronary flow on coronary perfusion pressure (CPP) and isovolumic systolic pressure (ISP) of Langendorff-perfused hearts under control conditions. Each point indicates the mean ± SEM (N = 7). *P<0.01 *vs* the smaller flow (one-way repeated measures ANOVA).

PE and the flow was then increased in steps according to the proposed protocol. A similar relationship between flow increment and CPP was obtained (Figure 2, upper panel). The same protocol was repeated perfusing the preparations with PE and NP and again similar results were obtained (Figure 2, upper panel).

When the correlation of coronary flow increments with ISP was determined (Figure 2, lower panel), the existence of a flow-dependent ISP increase was observed. However, during PE infusion ISP increased, displacing upwards the flow *vs* ISP curve. The infusion of PE + NP produced an additional increment of ISP values, again displacing upwards the flow *vs* ISP curve (Figure 2, lower panel).

Since the volume of the intraventricular balloon was maintained constant during these interventions, the effects of increasing coronary flow on ventricular diastolic pressure were analyzed. Although significant, only small increases of diastolic pressure were observed (Figure 3). It is interesting to note that as the coronary flow increased, left ventricular diastolic pressure (LVDP) also increased in controls and PE + NP perfused hearts. Preparations perfused only with PE did not show changes in LVDP with increasing flow.

## Discussion

In the present study we report evidence that during $\alpha_1$-adrenergic stimulation the mechanical performance may increase as the result of two factors simultaneously: a) direct $\alpha_1$-adrenergic stimulation of the myocardium and b) increased coronary perfusion pressure produced by the higher arterial blood pressure consequent to vasoconstriction.

Several investigators have described a positive inotropic effect produced by an increase in coronary perfusion pressure (1,2, 4,5). The putative mechanisms responsible for this positive inotropic effect were reported to be an increase of flow in excess of metabolic demands (2), sarcomere stretching as the result of distended vessels (7), a direct effect of pressure increasing intracellular calcium (14), and also as the result of changes in ionic composition or volume of the interstitium and an inotropic factor produced by the endothelium or intramyocardial neurons (6).

Under physiological conditions, a possible mechanism that increases coronary blood flow is an increase of perfusion pressure (5). Increasing peripheral vascular resistance can achieve this, for instance with $\alpha_1$-adrenergic stimulation, which, in turn, increases aortic pressure and CPP. In parallel, $\alpha_1$-adrenergic stimulation also increases force development. This effect is not mediated by increasing transmembrane $Ca^{2+}$ influx but by increasing the intracellular con-

centration of inositol (1,4,5)-trisphosphate ($IP_3$) (9-11). This seems to be achieved by activation of a phospholipase C that acts on phosphatidylinositol 4,5-biphosphate ($PIP_2$) forming $IP_3$ and diacylglycerol (DAG). $IP_3$ in turn activates sarcoplasmic reticulum $Ca^{2+}$ channels increasing myoplasmic $Ca^{2+}$ concentration (11,12).

Therefore, increased myocardial contraction may be obtained not only by the direct $\alpha_1$-adrenergic action on the myocardium but also by the increase in coronary perfusion pressure and flow resulting from a higher arterial blood pressure. We then investigated the contribution of each factor to the increase in myocardial contraction that occurs during $\alpha_1$-sympathetic stimulation.

The first protocol was used to investigate the $\alpha_1$-adrenergic stimulation produced by PE administration. This was done before and after simultaneous ß-adrenergic and cholinergic blockade since it is necessary to take into consideration that the existence of operational reflex mechanisms may superimpose or blunt this $\alpha_1$-adrenergic stimulation *in vivo*. The results showed that $\alpha_1$-adrenergic effects alone produced changes in LVSP and MAP both before and after the double blockade, but the cardiac inotropic state evaluated by dP/dt changes was not influenced by $\alpha_1$-adrenergic stimulation. The reduction in LVSP after the double blockade was expected (15). The lack of dP/dt enhancement during $\alpha_1$-adrenergic stimulation, despite an increase in LVSP, is usually found *in vivo* (16). Nevertheless, under more controlled conditions using an isolated preparation, $\alpha_1$-adrenergic stimulation alone increased the rate of force development or shortening (17). It is clear from these experiments that α-adrenergic stimulation increases LVSP and MAP. However, this increase in LVSP could be the result of a direct $\alpha_1$-adrenergic effect on the myocardium or the result of an increased coronary flow produced by the increased arterial blood pressure.

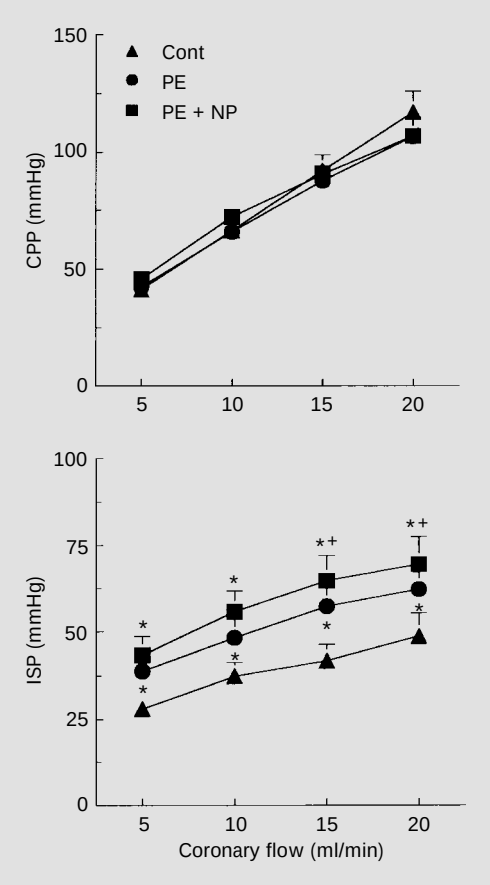


Figure 2 - *Upper panel*, Effects of increases of coronary flow on the coronary perfusion pressure (CPP) obtained under control conditions (Cont), during phenylephrine (PE) perfusion and during simultaneous perfusion of PE plus nitroprusside (NP). *Lower panel*, Effects of increases of coronary flow on the isovolumic systolic pressure (ISP) obtained under control conditions (Cont), during PE perfusion and during simultaneous perfusion of PE plus NP. Each point indicates the mean ± SEM (N = 7). *P<0.01 for PE or PE + NP *vs* the control condition at each flow. $^{+}$P<0.01 for comparisons between PE and PE + NP at each flow (two-way repeated measures ANOVA). Observe that CPP, although increasing as a function of flow increment, did not change with PE or PE + NP treatments.

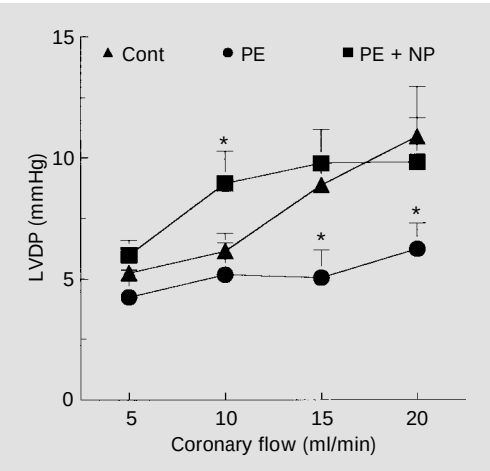


Figure 3 - Effects of increases of coronary flow on left ventricular diastolic pressure (LVDP) during phenylephrine (PE) perfusion and during simultaneous perfusion of PE plus nitroprusside (NP). Each point indicates the mean ± SEM (N = 7). *P<0.01 for PE or PE + NP *vs* control condition at each flow (repeated measures ANOVA).

*In vivo* $\alpha_1$-adrenergic stimulation increases systolic pressure by a direct myocardial effect and increases vascular resistance. The result is an increase of MAP and consequently of aortic pressure that increases CPP. We used Langendorff-perfused rat hearts to

evaluate the relative contributions of each factor, the increase in coronary flow produced by the increase in CPP and the myocardial $\alpha_1$-adrenergic stimulation.

The hearts were perfused at a constant flow since with this method perfusion pressure changes as a function of flow and vascular resistance. We chose to work with increasing flow rates because they simultaneously produced an increase in perfusion pressure. The relationship between flow and CPP obtained under control conditions and during PE or PE + NP perfusion was similar, suggesting that the changes in coronary vascular resistance were not demonstrable in the presence of the effects of the drugs.

The results indicate that the increase in coronary flow and perfusion pressure did increase ISP, in agreement with previous reports (1,2,5). The hearts were then perfused with PE, an $\alpha_1$-adrenergic agonist. It could be seen that LVSP was increased at all flow rates used, as suggested by the upward displacement of the pressure-flow curves. This indicates that an additional increase of pressure development was obtained, although the same relationship between pressure and flow was maintained. Since $\alpha_1$-adrenoceptor stimulation may produce coronary vasoconstriction (16) NP was used to fully dilate the vessels. On the basis of these findings, we conclude that, in addition to the ISP increase produced by increasing coronary flow, PE infusion produced an additional increase of ISP. This effect was increased by NP infusion, which produced an extra ISP increase. This would suggest that vasoconstriction could be limiting the availability of $O_2$. However, the existence of a similar CPP *vs* coronary flow relationship in the 3 conditions studied suggested that this is unlikely. The extra ISP increase could be explained by a small edema development as suggested by Rubboli et al. (18). In our preparations isolated hearts were perfused at 31°C and the solution was thoroughly filtered (0.8-µm filter) in order to reduce the increase in CPP during the course of the experiments (19). Nevertheless, a small increase in CPP and consequently ISP could be explained by edema formation (18). This view was supported by the fact that in our preparations the DP increased more during PE + NP infusion.

In conclusion, our data suggest that both mechanisms, i.e., $\alpha_1$-adrenoceptor stimulation and the flow increase produced by the higher arterial blood pressure consequent to peripheral vasoconstriction, can increase cardiac performance by acting simultaneously and synergistically.

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