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# THE INFLUENCE OF·EXPERIMENTAL HYPERLIPIDEMIA ON THE TIME COURSE OF CONTRACTILITY DURING SIMULATED ISCHAEMIA AND REPERFUSION AND RESPONSIVENESS TO PHENYLEPHRINE OF RAT HEART PAPILLARY MUSCLE

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The aim of this study was to examine the influence of simulated ischaemia on the contractility and responsiveness to phenylephrine of rat isolated papillary muscle in standard diet fed (SD) and hyperlipidemic diet fed (HLD) rats. The following parameters were measured: force of contraction (Fc), rate of rise ($+dF/dt$) and rate of fall ($-dF/dt$) of force of contraction, time to peak contraction (ttp) and relaxation time at 10% of total amplitude of contraction ($tt_{10}$). The baseline values of Fc and $+dF/dt$, but, not $-dF/dt$, were significantly lower in HLD group than in SD group. Tissues from HLD rats were more sensitive to ischaemia regarting Fc, $+dF/dt$ and $-dF/dt$. Moreover, reprefusion completely reversed the effects of ischaemia only in SD rats, but not in HLD rats, regarding Fc and $+dF/dt$. In contrast, a recovery of $-dF/dt$ during reperfusion occurred only in the HLD group. In SD rats, phenylephrine (10 and 30 µM) had no effect on the contractility or induced megative inotropic effects (100 and 300 µM). Propranolol (1 µM), a non-selective blocker of β-adrenoceptors, had no effects on this action. Chloroethylclonidine (CEC) (1 µM), a selectivw blocker of $\alpha_{1b}$-adrenoceptor subtype, but not WB-4101(2-((2,6-dimethoxyphenoxyethyl)amino-methyl-1,4-benzodioxane), a selective blocker of $\alpha_{1a}$ adrenoceptor subtype, abolishes the negative inotropic action of phenylephrine. In HLD rats, phenylephrine had positive inotropic action (10 and 30 µM).
The results indicate that hyperlipidemic diet in rats leads to the suppression of force of contraction and velocity of contraction, but not velocity of relaxation of isolated heart muscle. Under such a condition, heart muscle is more sensitive to ischaemia, but has better responsiveness to phenylephrine after ischeamia-reperfusion period.

K e y   w o r k s: *simulated ischaemia; experimental hyperlipidemia; heart contractility; $\alpha_1$-adrenoceptors.*

## INTRODUCTION

Correlation between serum cholesterol concentration and long-term risk of death from coronary heart disease (CHD) is well established (1). Moreover, in experimental atherosclerosis have been claimed hypersensitivity to

vasoconatrictors and increase in number of α-adrenoceptors (2). Recent oublished data have shown that some fatty acids can prevent ischaemia-induced malignant cardiac arrhythmias which is probably due to the suppression of voltage-gated L-type $Ca^{2+}$ currents (3). Although it is not clear whether hyperlipidemia just increases the risk of the development of CHD within a process of atherosclerosis, or directly changed some myocardium characteristics. So far, however, there has been no demonstration of the influence of high cholesterol and triglycerides levels on the heart contractility. Furthermore, the sensitivity of heart muscle, obtained from hyperlipidemic diet fed animals, to simulated ischaemia under *in vitro* condition, with a precise analysis of the mechanical characteristics of heart muscle has not been determined till now. Thus, the aim of our study was to establish the hyperlipidemic diet and to compare the mechanical characteristics of isolated heart muscle from standard diet and hyperlipidemic diet fed rats. We have compared an amplitude of force of contraction (Fc), velocity of contraction ($+dF/dt$) and velocity of relaxation ($-dF/dt$), time to peak contraction (ttp) and time of relaxation at the level of 10% of total force amplitude ($tt_{10}$) as well as a sensitivity to simulated ischaemia and reperfusion and a responsiveness of the heart muscle to phenylephrine. Phenylephrine, an $\alpha_1$-adrenoceptor agonist was used, as a functional test of the state of this population of receptors, in the light of earlier published data showing its hypersensitivity and an increasing in its number in myocardium under such a condition (2, 5, 6).

## MATERIALS AND METHODS

### Animals

Albino-Wistar rats of either gender, weighing 180—220 g were used. The animals were divided in two experimental groups: 1) standard diet fed rats (SD) and 2 ) hyperlipidemic died fed rats (HLD). The rats were housed in mesh-wire bottom cages (one animal in one cage) and kept in standard laboratory conditions (12 h light-dark cycle, 21°—24°C, humidity 50%—55%), with food (Murigran chow pellets, Bacutil Motycz, Poland) and tap water *ad libitum*. Additionally, HLD rats were fed the hyperlipidemic diet, containing (in g/kg): butter 400, casein 200 and nutrients. The diet (25 g/kg) was given daily in the morning for 30 days. After this period of time, it has been performed the measurement of the blood levels of cholesterol and triglycerides in HLD and SD groups, using commercial biochemical methods.

### Experimental procedures

After 30 days of diet, rats were anaesthetised with pentobarbital (i.p., 60 mg/kg), the thorax was opened, the heart quickly removed and placed in the preparation dish with modified, ice-cold Krebs-Henseleit solution (KHs), aerated with carbogen, where the left ventricle papillary muscles were prepared. After preparation, papillary muscle (length > 3 mm, diam. < 1 mm) was mounted

in 2 ml organ bath (Steiert Organ bath, type 813 with DC temperature controller type 319, HSE, Germany) and attached to an isometric force transducer (F-30, HSE, Germany). Isolated tissue was superfused with HKs containing (mM): NaCl 120.4; $CaCl_2$ 2.5; KCl 4.9; $MgCl_2 \times 6 H_2O$ 0.6; $NaH_2PO_4 \times H_2O$ 1.0; $NaHCO_3$, 15.3, glucose 11.5 and Na-pyruvate 2.0. The rate of perfusion was about 7 ml/min (peristaltic pump, type 371, Unipan, Poland). Solution was aerated with 95% $O_2$ and 5% $CO_2$, at 37°C $\pm$ 0.5°C. Resting tension was carefully adjusted to obtain the maximum force of contraction and was 0.4 $\pm$ 0.12 mN, n = 12. Tissues were electrically paced by two silver electrodes in contact with the mucles, with square waves, 0.5 Hz, 3 ms duration, threshold voltage + 20%, generated by an electronic atimulator (ST-02, Experimetria, Hungary). The developed tension (Fc), velocity of contraction (+dF/dt) and relaxation (−dF/dt) were measured by an isomwtric force-displacement transducer F-30 and bridge amplifier with a differenciator type 336 (HSE, Germany). The signals were displayed on a digital storage oscilloscope (VC-6525, Hitachi, Japan) and personal computer (PC 486) wit the HIMES software (Hitachi, Japan) allowing the measurement of time to peak and relaxation time of contraction (ttp and $tt_{10}$ respectively).

## Experimental protocol

All muscles were equilibrated for 60 min in oxygenated Krebs-Henseleit solution. Simulated ischaemia was achieved by superfusion of the tissue with no-substrate solution, gassing with $N_2$ 95%/$CO_2$ 5%, for 45 min. Instead substrate (glucose and Na-pyruvate), 7.0 mM choline chloride was added to the hypoxic solution (7). Reperfusion was achieved by switching from no-substrate, hypoxic solution to oxygenated KHs solution for 60 min. Then, phenylephrine was added in rising concentrations. The measurement of Fc, +dF/dt, −dF/dt, ttp and $tt_{10}$ were performed after period of equilibration, after 10, 20, 30 and 45 min of ischaemia, after 10, 20, 30 and 60 min reperfusion and after 10 min of perfusion with every concentration of phenylephrine, phenylephrine in the presence of propranolol, phenylephrine in the presence of corynanthine, phenylephrine in the presence of WB-4101 and phenylephrine in the presence of chloroethylclonidine. Six preparations from both SD and HLD groups were incubated for 60 min plus 45 min pluc 60 min in normal, oxygenated solution, without ischaemia. Then, phenylephrine was added. It was a control group regarding the inotropic effects of phenylephrine.

## Statistics

Data are expressed as means $\pm$ s.e.m. Differences between control values and means at different time course of ischaemia and reperfusion means of corresponding values in SD and HLD groups during ischaemia and reperfusion, corresponding values obtained with the same concentration of phenylephrine in different experimental groups were evaluated using two-way analysis of variance (ANOVA) following by a Newman-Keuls test. The computer program Pharmacological Calculation System Pharm/PCS, version 4 based on the Manual of pharmacologic calculations with computer programs (7) was used. $P < 0.05$ was considered to be statistically significant.

## Drugs

All components used in the preparation of the solutions were from Sigma. Phenylephrine hydrochloride, corynanthine and WB-4101 (2-((2,6-dimethoxyphenoxyethyl)amino-methyl)--1,4-benzodioxane) were from Sigma. Propranolol and chloroenthylclonidine (CEC) were from RBI, Natick, USA. The used drugs were dissolved in distilled water.

<div align="center">RESULTS</div>

*Effects of diet on the cholesterol and triglyceride serum level*

Total serum cholesterol and serum triglyceride concentrations were determined in 8 animals from hyperlipidemic diet fed rats (HLD) and 8 animals from standard died fed rats (SD), after 30 days of diet, using commercial biochemical tests. It has been found a significant increase either in level of cholesterol ($63.4 \pm 3$ mg/dl in SD group vs $82.3 \pm 6$ mg/dl, $P < 0.05$) or triglyceride ($62.6 \pm 13$ mg/dl vs $120.5 \pm 22.5$ mg/dl, $P < 0.01$).

*Effects of hyperlipidemic diet on the baseline parameters of contractility*

As *Fig. 1* shows, force of contraction (Fc) and velocity of contraction ($+dF/dt$), but not velocity of relaxation ($-dF/dt$) were significantly lower in HLD rats. Duration of contraction and relaxation of right ventricle papillary muscle obtained from SD rats was almost identical, $64.2 \pm 2.5$ ms and $69.3 \pm 4.5$ ms, respectively. In contrast, duration of relaxation of isolated papillary muscles obtained from HLD rats was significantly higher than duration of contraction ($64 \pm 3.6$ ms vs $49.2 \pm 3$ ms, n = 6, $P < 0.05$).
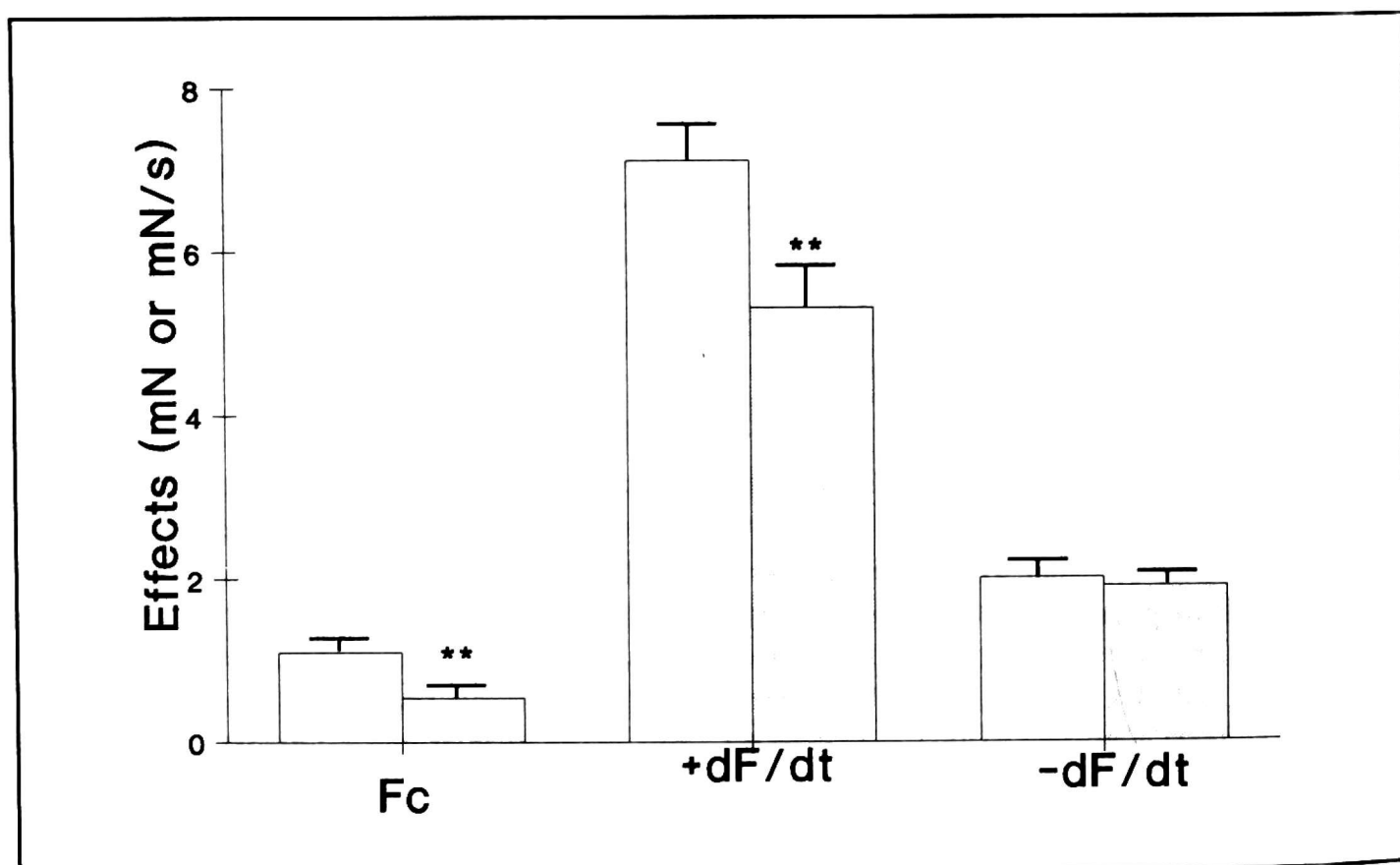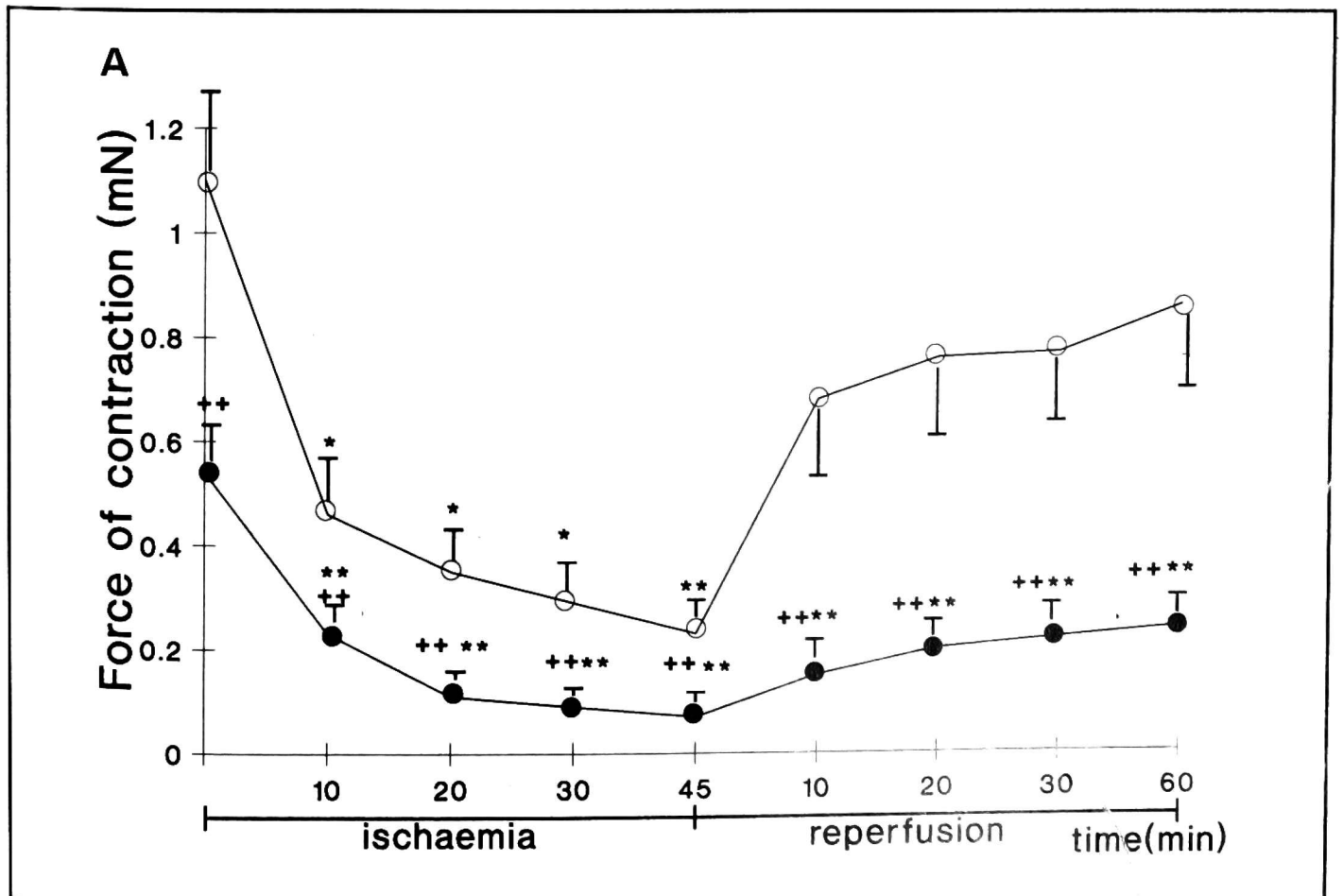


*Fig. 1.* The baseline values of force of contraction (Fc), velocity of contraction ($+dF/dt$) and velocity of relaxation ($-dF/dt$) in papillary muscles obtained from standard diet (SD) and hyperlipidemic diet fed rats (HLD). Light bar — SD rats, Dark bar — HLD rats. ** $P < 0.01$; significant difference between corresponding values, mean $\pm$ S.E.M., n = 6, twotailed Student *t*-test, grouped data.

*Effects of simulated ischaemia and reperfusion on the contractility of isolated right ventricle papillary muscle obtained from SD and HLD rats*

*Fig. 2a, 2b* and *2c* depict the time-course changes in force of contraction, velocity of contraction and velocity of relaxation, respectively, during ischaemia and reperfusion. We have compared the differences with respect to the control values, but also between corresponding values at the same time points. It can be seen that simulated ischaemia induced significantly stronger decreases of Fc, $+dF/dt$ and $-dF/dt$ in HLD group than in SD group. What is more, reperfusion caused a complete recovery of Fc and $+dF/dt$ in SD rats, but not in HLD rats. In contrast, the fall in velocity of relaxation ($-dF/dt$) during ischaemia was completely reversed during reperfusion only in HLD rats (*Fig. 2c*). *Table 1* shows that time to peak contraction (ttp) was shortened during ischaemia in the similar extent in both experimental groups, SD and HLD, without significant differences between corresponding values. In contrast, relaxation time at the level of 10% of total amplitude of contraction ($tt_{10}$) was not changed significantly. Taking into account that ttp and $tt_{10}$ duration are dependent not only on the velocity of contraction and relaxation, but on the amplitude of force too, we have compared the significance between the $ttp/tt_{10}$ ratio at all time points during simulated ischaemia/reperfusion period. Nevertheless, it has not been found any significant differences (data not shown).
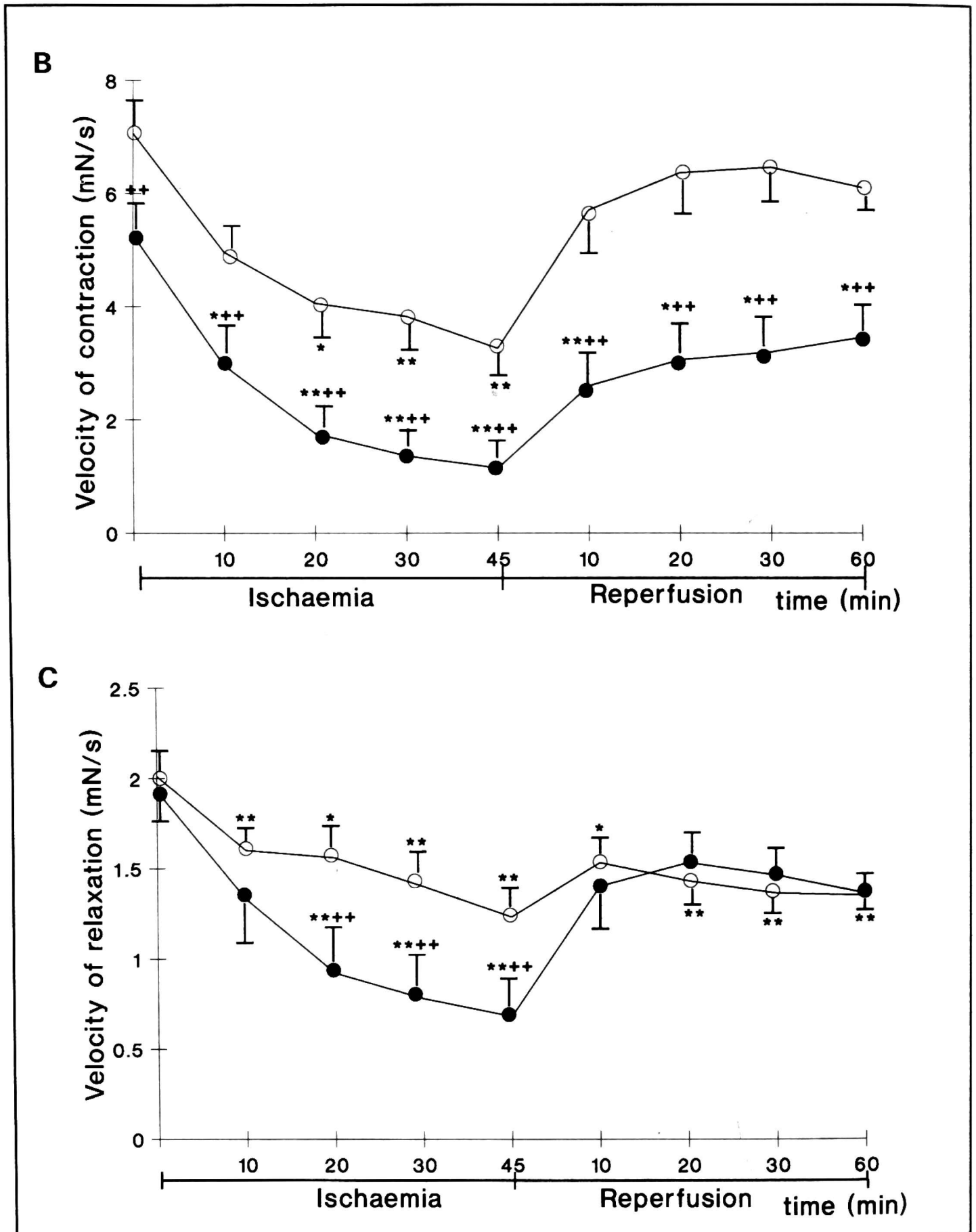
*Fig. 2.* The time-course of force of contraction (2 a), velocity of contraction (2 b) and velocity of relaxation (2 c) in isolated papillary muscles obtained from standard diet fed (SD) and hyperlipidemic diet fed (HLD) rats, during simulated ischaemia and reperfusion periods. o — SD group, ● — HLD group, * $P < 0.05$; ** $P < 0.01$; significant differences with respect to the baseline value. Mean ± S.E.M., n = 6 experiments, [+]$P < 0.05$; [++]$P < 0.01$; significant differences between corresponding values at the same time-points. Mean ± S.E.M., from six experiments. Newman-Keuls test following two-way ANOVA.

*Table 1.* The influence of simulated-ischaemia and reperfusion on the duration of contraction (ttp) and relaxation at 10% of total amplitude ($tt_{10}$) in papillary muscles of standard diet (SD) and hyperlipidemic died fed (HLD) rats

| Time (min) | SD ttp (ms) | HLD ttp (ms) |
|---|---|---|
| control | $64.2 \pm 2.5$ | $49.2 \pm 3$ |
| ischaemia | | |
| 10 | $54 \pm 2.5$ [a] | $36 \pm 4.7$ [b] |
| 20 | $48.7 \pm 1.8$ [b] | $34.8 \pm 4.4$ [b] |
| 30 | $40.5 \pm 2.8$ [b] | $29.5 \pm 4$ [b] |
| 45 | $43.8 \pm 2.2$ [b] | $27.6 \pm 4.6$ [b] |
| reperfusion | | |
| 10 | $55.2 \pm 1.7$ | $42.5 \pm 3.6$ |
| 20 | $56.8 \pm 1.4$ | $43.5 \pm 2.7$ |
| 30 | $51.5 \pm 2.8$ [a] | $39.5 \pm 3.2$ [b] |
| 60 | $51 \pm 2.2$ [a] | $41.5 \pm 2.3$ [a] |
| | SD $tt_{10}$ ms) | HLD $tt_{10}$ (ms) |
| control | $69.3 \pm 4.5$ | $64 \pm 3.6$ |
| ischaemia | | |
| 10 | $69.8 \pm 2.3$ | $51.2 \pm 6.7$ |
| 20 | $55.3 \pm 2$ | $48.8 \pm 6.9$ |
| 30 | $68.2 \pm 5$ | $43.5 \pm 6$ |
| 45 | $57.2 \pm 6.1$ | $39.8 \pm 6.5$ |
| reperfusion | | |
| 10 | $63.5 \pm 5.2$ | $54.3 \pm 4.8$ |
| 20 | $64.6 \pm 7$ | $49.8 \pm 4.5$ |
| 30 | $70.2 \pm 5.5$ | $48.5 \pm 2.8$ |
| 60 | $62.8 \pm 1.5$ | $44.6 \pm 2.8$ |

[a] $P < 0.05$; [b] $P < 0.01$; significant differences with respect to the control values in each group. Means $\pm$ S.E.M. from six experiments. One way ANOVA followed with Newman-Keult test.

*Effects of phenylephrine on the contractility of isolated rat papillary muscle obtained from SD and HLD groups*

*Fig. 3* presents the effects of phenylephrine on the force of contaction cf isolated papillary muscles obtained from SD group which was not treated with simulated ischaemia (control), obtained from SD group, but treated with simulated ischaemia/reperfusion procedure and papillary muscles obtained from HLD group not treated and treated with simulated-ischaemia reperfusion procedure. In order to illustrate better the differences in phenylephrine effects between SD and HLD preparationsm the results were presented as the changes in force expressed in % of control. It can be seen a lack of effects of low concentration of phenylephrine in the muscles obtained from SD rats after ischaemia/reperfusion period. For instance, the same concentrations of phenylephrine induced positive inotropic action in the muscles obtained from

HLD rats, even significantly higher than in muscles not treated with ischaemia obtained from SD rats and similar to that obtained from HLD rats before ischaemia. At higher concentration, phenylephrine induced negative inotropic action in both HLD and SD muscles treated with ischaemia, but this effect was significantly weaker in HLD rats.
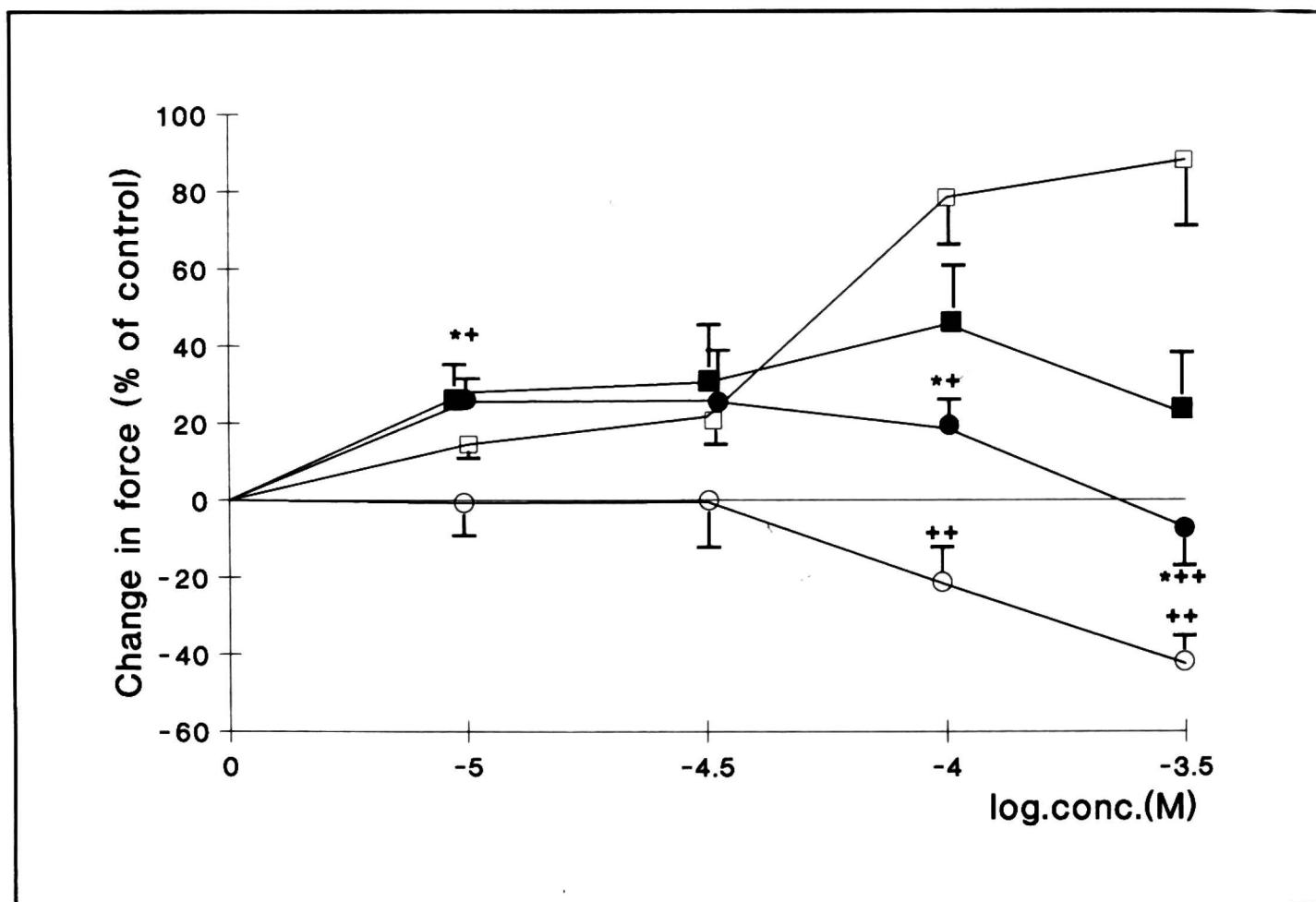


Fig. 3. The effects of phenylephrine on the contractility of isolated papillary muscles non-treated with ischaemia-reperfusion procedure in SD rats (control-SD), non-treated with ischaemia--reperfusion procedure in HLD rats (control-HLD), after ischaemia-reperfusion in standard diet fed rats (SD), and after ischaemia-reperfusion in hyperlipidemic diet fed rats (HLD). □ — control-SD, ■ — control-HLD, o — SD rats, ● — HLD rats, [+]P < 0.05; [++]P < 0.01; significant differences with respect to the corresponding values of control-SD group; *P < 0.05; significant differences between corresponding values of SD and HLD groups. Mean ± S.E.M. from six experiments, two-way ANOWA with Newman-Keuls test.

In order to clarify the mechanism of this negative inotropic action of phenylephrine, we have performed additional experiments with phenylephrine in muscles obtained from SD rats after ischaemia/reperfusion in the presence of 1 μM of propranolol or 1 μM of corynanthine, or 1 μM of CEC, or 1 μM of WB-4101. The reason for using propranolol, a non-selective blocker of β-adrenoceptor was to exclude an involvement of these type of receptor in the negative inotropic action of phenylephrine presented here. Corynanthine is

a selective blocker of $\alpha_1$-adrenoceptor, CEC is a selective blocker of $\alpha_{1b}$-adrenoreceptor subtype and WB-4101 is a selective blocker of $\alpha_{1a}$ adrenoceptor subtype. As *Fig. 4* shows, corynanthine and CEC but not propranolol and WB-4101, completely reversed the negative inotropic effect of phenylephrine. It is noteworthy that none of above mentioned antagonists have had a significant effect on the contractility of isolated papillary muscle in concentration used here.
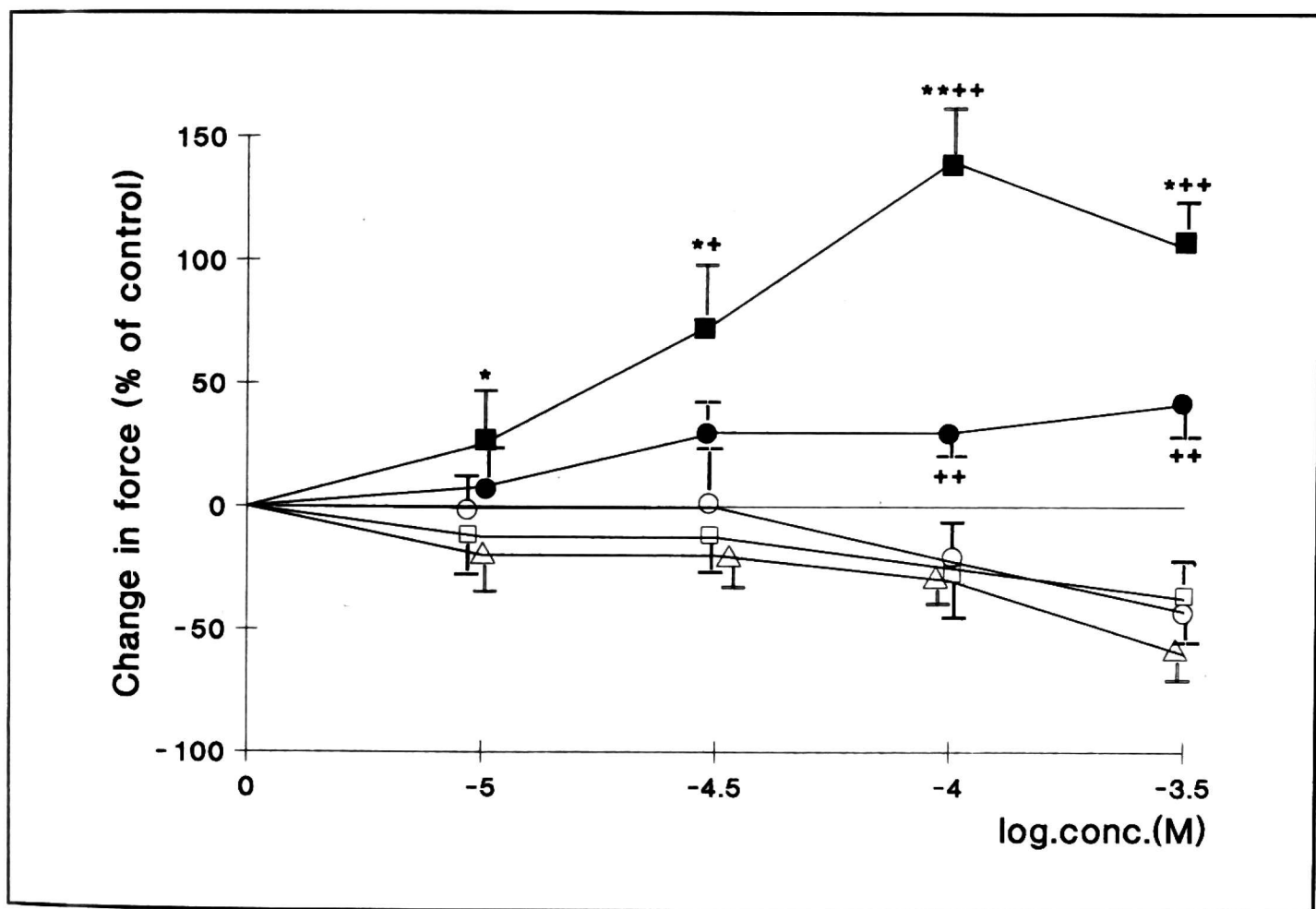


*Fig. 4*. The effects of corynanthine (1 μM), propranolol (1 μM), chloroethylclonidine (CEC) (1 μM) and WB-4101 (1 μM) on the phenylephrine inotropic action in standard diet fed rats, after simulated ischaemia and reperfusion periods. o — effects of phenylephrine alone, ● — effects of phenylephrine in the presence of corynanthine, □ — effects of phenylephrine in the presence of propranolol, ■ — effects of phenylephrine in the presence of CEC, ∇ — effects of phenylephrine in the presence of WB-4101, $^+P < 0.05$, $^{++}P < 0.01$; significant differences with respect to the values obtained with phenylephrine alone; $*P < 0.05$, $**P < 0.01$, significant differences with respect to the baseline value obtained before phenylephrine was added. Mean $\pm$ S.E.M. from four experiments, two-way ANOWA with Newman-Keuls test.

*Table 2* summarised the effects of phenylephrine on the velocity of contraction and relaxation. It can be seen that the velocity of contraction in the muscles obtained from HLD rats was significantly higher than in SD group treated with ischaemia. On the contrary, no significant changes in velocity of relaxation were observed.

*Table 2.* Changes in velocity of contraction (+dF/dt) and relaxation (−dF/dt) induced by phenylephrine in rat papillary muscles obtained from control group (without ischaemia), SD group and HLD group (after simulated ischaemia/reperfusion period), expressed as a % of baseline values obtained before drug addition

| Phenylephrine | +dF/dt | | | −dF/dt | | |
|---|---|---|---|---|---|---|
| | control | SD | HLD | control | SD | HLD |
| Baseline values (mN/s) | $6.7 \pm 0.27$ | $7.1 \pm 0.5$ | $5.3 \pm 0.5$ | $1.9 \pm 0.6$ | $2 \pm 0.1$ | $1.9 \pm 0.2$ |
| Concentrations ($\mu$M) | | | | | | |
| 10 | $7.4 \pm 0.8$ | $-0.3 \pm 2^a$ | $9.5 \pm 1.8^d$ | $-0.4 \pm 5$ | $-2.5 \pm 7.3$ | $9.1 \pm 3.7$ |
| 30 | $-1.5 \pm 5.8$ | $-3.5 \pm 4.3$ | $7.4 \pm 2.5^c$ | $2 \pm 5.3$ | $-6 \pm 5.6$ | $5 \pm 4.6$ |
| 100 | $25.7 \pm 8.5$ | $-11.4 \pm 3.7^b$ | $10 \pm 4^d$ | $3.3 \pm 9$ | $-6 \pm 5.6$ | $6.2 \pm 3.8$ |
| 300 | $26.9 \pm 10$ | $-24 \pm 4.7^b$ | $-8.1 \pm 10^a$ | $15.3 \pm 18$ | $-4 \pm 6.8$ | $-2.2 \pm 6.3$ |

[a] P < 0.05; [b] P < 0.01; significant differences with respect to the control values; [c] P < 0.05; [d] P < 0.01; significant differences between corresponding values of SD and HLD groups. Means ± S.E.M. from six experiments. Two-way ANOVA with Newman-Keults test.

## DISCUSSION

The major findings of this study are that papillary muscles obtained from HLD rats have shown the lower force of contraction and velocity of contraction, higher sensitivity to ischaemia and different response to phenylephrine after ischaemia/reperfusion period then the muscles obtained from SD rats. It is notewortha that velocity of relaxation, decreased during ischaemia, returned to its control value after reperfusion only in HLD group. Moreover, phenylephrine had significant negative inotropic action only in SD group of rats, while its effects before and after simulated ischaemia/reperfusion period in HLD rats were not significantly different There are several points requiring an explanation in the light of above mentioned results: 1) Whey the baseline values of force of contraction and velocity of contraction, but not velocity of relaxation are significantly lower in HLD group? 2) Why the contractility of papillary muscles from HLD rats is more sensitive to ischaemia? 3) Why the velocity of relaxation recovered to its control value after ischaemia-reperfusion period only in HLD rats? 4) What is the mechanism of negative inotropic action of phenylephrine, occurring only in SD group?

The effects of a hyperlipidemic diet on the heart contrictility should be clarified. It has been demonstrated the correlation between high cholesterol concentration in serum with risk of coronary heart disease (CHD) (9). Also, there is an evidence for significant positive associations between the incidence of CHD and serum cholesterol level in younger population (10). Recent data have shown that polyunsaturated fatty acids suppress the voltage-gated L-type $Ca^{2+}$ channels in adult and neonatal rat (3). Howeverm a little is known about

direct influence of cholesterol or triglycerides on the heart contractility or adrenergic responsiveness of myocardium. This study demonstrates that the papillary muscles obtained from rats fed hyperlipidemic diet have significantly weaker force and velocity of contraction, but not velocity of relaxation compared with standard diet fed rats (SD group). The diet which we used in this study induced significant rise of the serum levels of cholesterol and specially triglycerides. Hence, above mentioned differences in contractility of the muscles from HLD and SD groups are probably due to extremely high level of triglycerides in HLD rats. The mechanism of this effect could involve an inhibition of voltage-gated $Ca^{2+}$ channels, however this action was demonstrated only in case of polyunsaturated fatty acids till now (3).

The next point requiring an analysis is the effect of ischaemia on the heart muscle. It is well known that ischaemia produces an increase in intracellular calcium in heart muscle. The pathophysiology of this calcium overload involved an increase in density of voltage dependent calcium channels (11). Next, the proteolysis of troponim I was observed, which can be responsible for a decrease of $Ca^{2+}$ responsiveness of the myofilaments in ischaemic myocardium (12). This study has demonstrated the higher sensitivity to ischaemia of the muscles obtained from HLD rats regarding Fc, +dF/dt and −dF/dt. However, reperfusion caused a complete recovery of Fc and +dF/dt only in SD group, and −dF/dt only in HLD group. The possible explanation for this differences could be the change in metabolism of heart muscle of HLD rats accustomed to high levels of cholesterol and triglycerides. But, it is well known that under ischaemic condition anaerobic metabolism with using of glucose dominates (12). The recovery of velocity of relaxation in HLD group, however, might be an evidence for smaller overloading with $Ca^{2+}$ in this group of preparation, in comparison with SD group. The different effects of phenylephrine on the contractility of papillary muscles from SD and HLD groups demands also an explanation. As our study has shown, phenylephrine, an $\alpha_1$-adrenoceptor agonist, exerted positive inotropic action in ischaemia-non treated muscles obtained from SD group, lack of effect or negative inotropic action in muscles obtained from SD rats, and potentiated positive inotropic effects at low concentrations in HLD group, either before or after ischaemia/reperfusion period. There is an evidence that myocardial ischaemia increases the number of $\alpha_1$-adrenoceptors in the heart muscle (6). However, it is not known which kind of $\alpha_1$ receptor subtypes is overexpressed during ischaemia. Data presented here, obtained in the presence of different antagonists of adrenergic receptors suggest that the overexpression of $\alpha_{1b}$ receptor subtype is responsible for the negative inotropic action of phenylephrine observed in SD group after ischaemia/reperfusion period. It is in accordance with literature data that this subtype of $\alpha_1$-adrenoceptors is responsible for a negative inotropic action of phenylephrine (14). One must

remember that we have recorded the effects of phenylephrine after 7 to 10 min of incubation with every concentration of phenylephrine, to avoide the transient effects of phenylephrine and to obtained only a sustained action (14). On the other hand, potentiation of positive inotropic action of phenylephrine at low concentrations and lack of significant negative inotropic action at higher concentrations in HLD group of preparations (see *Fig. 3* and *Fig. 4*) indicates that hyperlipidemia prevents this overexpression. However, the precise mechanism of this phenomenon remains to be clarify.

To conclude, this study has shown that the diet which was used in this study induces significant increase in the serum level of triglycerides and cholesterol in rats and modifies the contractility and responsiveness to phenylephrine of isolated papillary muscles. The main difference between SD and HLD preparations is complete recovery of velocity of relaxation after ischaemia/reperfusion period only in HLD group and negative inotropic action of phenylephrine, reversed by CEC, only in SD group. It suggests that hyperlipidemic diet prevents an overexpression of $\alpha_{1b}$-adrenoceptor subtype during ischaemia.

## REFERENCES

1. Shekelle RB, Shryock AM, Paul O *et al.* Diet, serum cholesterol, and death from coronary heart disease. The Western Electric Study. *N Engl J Med* 1981; 304: 65—70.
2. Nanda V, Henry PD. Increased serotonergic and alpha adrenergic receptors in aorta from rabbits fed a high cholesterol diet. *Clin Res* 1982; 30: 209A.
3. Xiao YF, Gomez AM, Morgan JP, Lederer WJ, Leaf A. Suppression of voltage-gated L-type $Ca^{2+}$ currents by polyunsaturated fatty acids in adult and neonatal rat ventricular myocytes. *Proc Natl Acad Sci USA* 1997; 94: 4182—4187.
4. Gresham GA, Howard AN. The independent production of atherosclerosis and thrombosis in the rat. *Br J Exp Pathol* 1960; 41: 395—402.
5. Rosendorff C, Hoffman JI, Verrier ED, Rouleau J, Boerboom LE. Cholesterol potentiates the coronary artery response to norephinephrine in anesthetized and conscious dogs. *Circ Res* 1981; 48: 320—329.
6. Li K, He H, Li C, Sirois P, Rouleau JL. Myocardial $\alpha_1$-adrenoceptor: inotropic effect and physiologic and pathologic implications. *Life Sci* 1997; 60: 1305—1318.
7. Walker DM, Marther MS, Walker JM, Yellon DM. Preconditioning in isolated superfused rabbit papillary muscles. *Am J Physiol* 1994; 266 (4Pt2): H1534—H1540.
8. Tallarida RJ, Murray RB. Manual of pharmacologic calculations with computer programs. New York, Springer-Verlag 1987; pp. 110—134.
9. Kushi LH, Lew RA, Stare FJ *et al.* Diet and 20-year mortality from coronary heart disease. The Ireland-Boston Diet-Heart Study. *N Engl. J Med* 1985; 312: 811—818.

10. Posner BM, Cobb JL, Belanger AJ, Cupples A, D'Agostino RB, Stokes J. Dietary lipid predictors of coronary heart disease in men. The Framingham Study. *Arch Intern Med* 1991; 151: 1181—1187.

11. Blanck TJJ, Yasukochi S, Quigg M, Curtis WE, Gardner TJ. Global ischemia increases the density of voltage-dependent calcium channels in porcine cardiac sarcolemma. *Anesth Analg* 1997; 84: 972—975.

12. Gao WD, Atar D, Liu Y, Perez ND, Murphy AM, Marban E. Role of troponin I proteolysis in the pathogenesis of stunned myocardium. *Circ Res* 1997; 80: 393—399.

13. Bristow JD, Arai AE, Anselone CG, Pantely GA. Response to myocardial ischemia as a regulated process. *Circulation* 1991; 84: 2580—1587.

14. Williamson AP, Seifen E, Lindeman JP, Kennedy RH. Effects of WB4101and chloroethylclonidine on the positive and negative inotropic actions of phenylephrine in rat cardiac muscle. *J Pharmacol Exp Ther* 1994; 268: 1174—1182.

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