

I. KOCIĆ, D. DWORAKOWSKA, R. DWORAKOWSKI

## 4-AMINOPYRIDINE INDUCES POSITIVE LUSITROPIC EFFECTS AND PREVENTS THE NEGATIVE INOTROPIC ACTION OF PHENYLEPHRINE IN THE RAT CARDIAC TISSUE SUBJECTED TO ISCHAEMIA

Department of Pharmacology, Medical University of Gdańsk, Poland

The effects of 4-aminopyridine (4-AP) at concentration of 1 mM on the contractility of rat isolated papillary muscle subjected to simulated ischaemia has been evaluated. Additionally, the effects of 4-AP on the phenylephrine inotropic action (a selective agonist of  $\alpha_1$ -adrenergic receptor) on rat isolated cardiac tissue underwent simulated ischaemia and reperfusion was studied. Experiments were performed on rat isolated papillary muscles obtained from left ventricle. The following parameters have been measured: force of contraction (Fc), velocity of contraction (+dF/dt), velocity of relaxation (–dF/dt) and the ratio between time to peak contraction (ttp) and relaxation time at the level of 10% of total contraction amplitude (tt<sub>10</sub>) as an index of lusitropic effects. Simulated ischaemia lasting 45 min was induced by replacement of standard normoxic solution by no-substrat one gassing with 95% N<sub>2</sub>/5%CO<sub>2</sub>. Although 4-AP exerted a slight, but significant positive inotropic action itself, pretreatment with 1 mM of this compound significantly depressed a recovery of Fc and +dF/dt, but improves recovery of –dF/dt in the rat papillary muscle during reperfusion as compared with control group of preparations. Moreover, the paradoxical negative inotropic action of phenylephrine observed in rat stunned papillary muscle was prevented in preparations previously treated by 4-AP. These findings suggest that an inhibition of outward K<sup>+</sup> current (probably transient outward and rapid component of delayed rectifying currents at 1 mM of 4-AP) aggravates ischaemia-induced failure in contractility but prevents changes in  $\alpha_1$ -adrenergic receptor signaling pathway occurring during ischaemia.

*Key words:* 4-aminopyridine; simulated ischaemia; papillary muscle;  $\alpha_1$ -adrenergic receptor

### INTRODUCTION

Experiments previously performed in this laboratory have shown that phenylephrine, a selective agonist of  $\alpha_1$ -adrenergic receptor, caused the negative inotropic action in rat isolated papillary muscle subjected to simulated ischaemia and reperfusion (1). On the other hand, the effects of

isoproterenol, an agonist of  $\beta$ -adrenoceptors, on the contractility of rat isolated cardiac tissues before and after subjection to ischaemia-reperfusion procedure were not significantly different. The negative inotropic action of phenylephrine in the rat stunned isolated papillary muscle was entirely reversed in the presence of 1 and 3  $\mu\text{M}$  of chloroethylclonidine, an irreversible and selective antagonist of  $\alpha_{1b}$ -adrenergic receptor subtype (1). Moreover, pretreatment of the cardiac muscles by glibenclamide, a selective blocker of ATP-sensitive  $\text{K}^+$  channels, and terikalant (1  $\mu\text{M}$ ), a blocker of inward rectifier  $\text{K}^+$  channels prevented the paradoxical negative inotropic action of phenylephrine in rat papillary muscle (2). Thus, the aim of this study was to extend previous investigation and to find out the effects of 4-aminopyridine, a blocker of transient outward  $\text{K}^+$  channels at concentration of 1 mM (3, 4), on the disturbances in contractility during ischaemia and on the effects of phenylephrine in preparations previously underwent ischaemia-reperfusion procedure.

## MATERIALS AND METHODS

### *Animals*

Albino-Wistar rats of either gender, weighing 180–220 g were used. 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*.

### *Experimental procedures*

Rats were anaesthetised with pentobarbital (i.p., 60 mg/kg), thorax was opened, 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 >3mm, 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 KHs containing (mM): NaCl 120.4;  $\text{CaCl}_2$  2.5; KCl 4.9;  $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  0.6;  $\text{NaH}_2\text{PO}_4 \times \text{H}_2\text{O}$  1.0;  $\text{NaHCO}_3$  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%  $\text{O}_2$  and 5%  $\text{CO}_2$ , at 37°C  $\pm$  0.5°C. Resting tension was carefully adjusted to obtain the maximum force of contraction and was 0.4  $\pm$  0.15 mN, n = 12. Tissues were electrically paced by two silver electrodes in contact with the muscles, with square waves, 0.5 Hz, 3 ms duration, threshold voltage +20%, generated by an electronic stimulator (ST-02, Experimetria, Hungary). The developed tension ( $F_c$ ), velocity of contraction (+dF/dt) and relaxation (–dF/dt) were measured by an isometric force-displacement transducer F30 and bridge amplifier with a differentiator type 336 (HSE, Germany). The signals were displayed on a digital storage oscilloscope (VC–6525, Hitachi, Japan) and personal computer (PC 486) with the HIMES software (Hitachi, Japan) allowing the measurement of time to peak and relaxation time of contraction ( $t_{tp}$  and  $t_{t_{10}}$  respectively).

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  $\text{N}_2$  95%/CO<sub>2</sub> 5%, for 45 min. Instead substrate (glucose and Napyruvate), 7.0 mM choline chloride

was added to the hypoxic solution (5). Reperfusion was achieved by switching from no-substrate, hypoxic solution to oxygenated KHs solution for 60 min. Then, phenylephrine was added in rising concentrations. It was a control group of experiments. Separate group of preparations were treated with the same procedure except previous addition of 4-AP, at concentration of 1 mM, for 20 min. The measurement of  $F_c$ ,  $+dF/dt$ ,  $-dF/dt$ ,  $t_{tp}$  and  $t_{t_{10}}$  were performed after period of equilibration, after 10, 20, 30 and 45 min of ischaemia, after 10, 20, 30 and 60 min of reperfusion and after 7 min of perfusion with every concentration of phenylephrine. Additionally,  $t_{tp}/t_{t_{10}}$  ratio was calculated as an index of lusitropic effects. The significant rise or fall of this ratio was considered as a positive or negative lusitropic effect, respectively. The reason for this is that velocity of relaxation or duration of relaxation taken as absolute values are not precise indicators of muscle lusitropism (ability of relaxation). These parameters are dependent on amplitude of force of contraction and only expression of the duration of relaxation related to the changes in the duration of contraction at the same time can be a measure of lusitropic ability of heart muscle. Six preparations were incubated for 60 min plus 45 min plus 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 and between corresponding values obtained with the same concentration of phenylephrine in different experimental groups (control group without ischaemia, after ischaemia and reperfusion or after pretreatment with 4-AP, ischaemia and reperfusion) were evaluated using one-way analysis of variance (ANOVA) followed by a Newman-Keuls test., or two-tailed Student t-test, paired data for the evaluation of inotropic effects of 4-AP. The computer program Pharmacological Calculation System Pharm/PCS, version 4 based on the Manual of pharmacologic calculations with computer programs (6) was used  $P < 0.05$  was considered to be statistically significant.

### Drugs

All components used in the preparation of the solutions, phenylephrine hydrochloride and 4-aminopyridine (4-AP) were from Sigma, St. Louis, USA. Propranolol was from RBI. The used drugs were dissolved in distilled water.

## RESULTS

*The effects of simulated ischaemia on the time course of contractility in the absence and in the presence of 4-AP in rat isolated papillary muscle*

*Fig. 1A* depicts the changes in  $F_c$ ,  $+dF/dt$  and  $-dF/dt$  during simulated ischaemia and reperfusion. Baseline values for the measured parameters for the first series of experiments in the absence of 4-AP were:  $F_c = 1.1 \pm 0.2$  mN,  $+dF/dt = 7.1 \pm 0.5$  mN/s,  $-dF/dt = 2.1 \pm 0.1$  mN/s,  $n = 6$ ; for the second series of experiments before an addition of 4-AP were:  $F_c = 0.8 \pm 0.2$  mN,  $+dF/dt = 5.7 \pm 1$  mN/s,  $-dF/dt = 2.0 \pm 0.3$  mN/s, and after an addition of 4-AP were:  $F_c = 1.1 \pm 0.3$  mN,  $+dF/dt = 6.2 \pm 0.8$  mN/s,  $-dF/dt = 3.3 \pm 0.4$  mN/s,  $P < 0.05$ ,  $n = 6$ . As *Fig. 1B* shows, the falls in  $F_c$ ,  $+dF/dt$

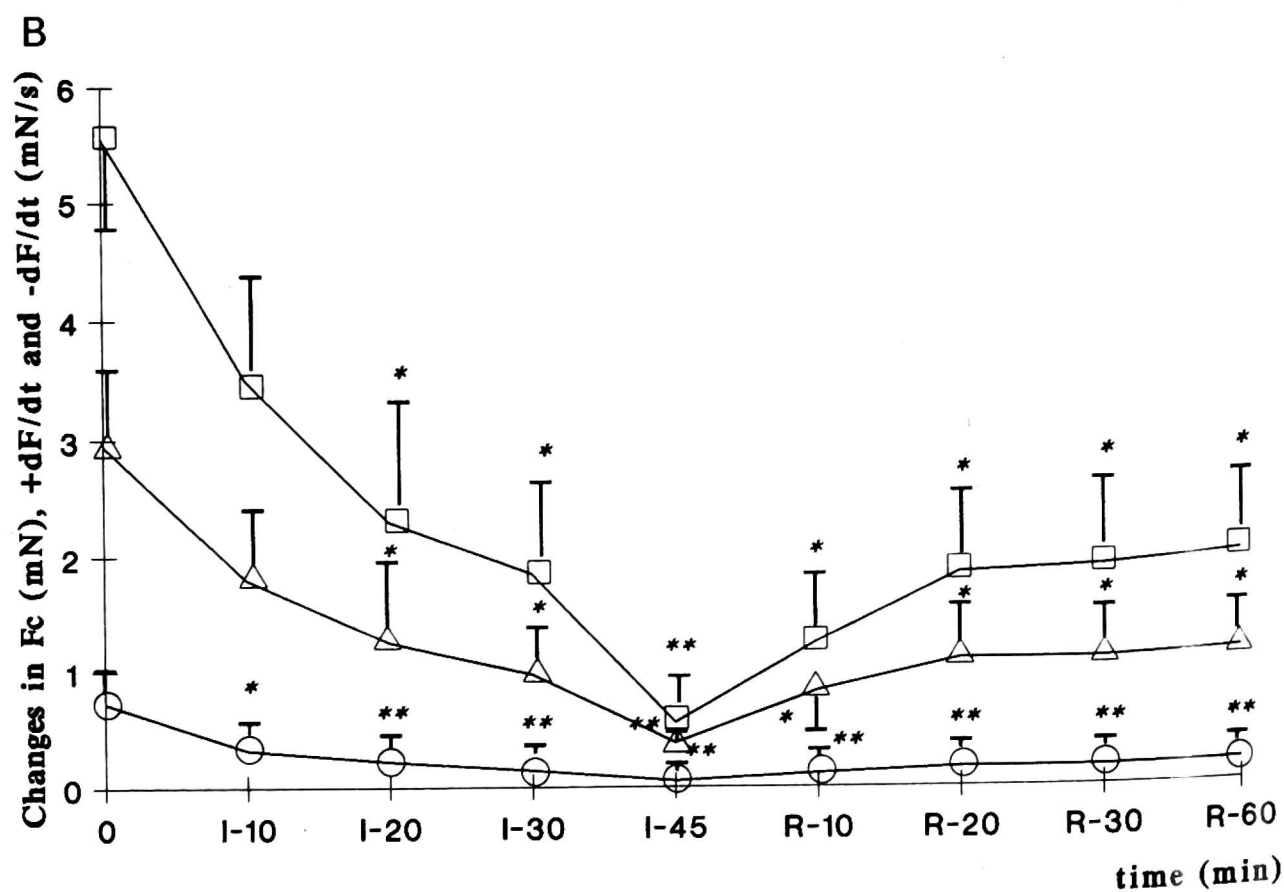
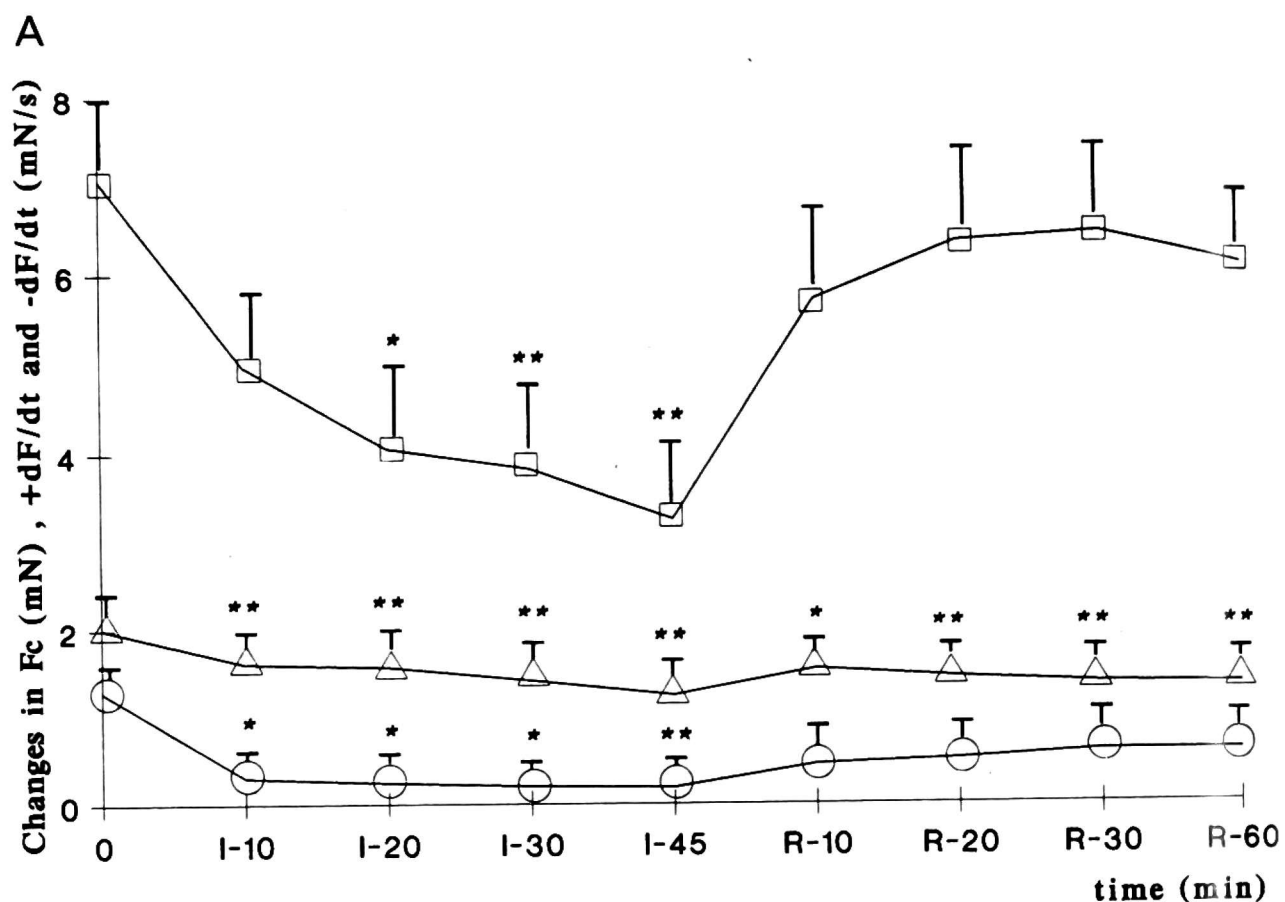


Fig. 1. The time course of force of contraction ( $F_c$ ), velocity of contraction ( $+dF/dt$ ) and velocity of relaxation ( $-dF/dt$ ) during simulated ischaemia/reperfusion in the absence (a) and in the presence of 1 mM of 4-AP (b).  $\circ$  —  $F_c$ ,  $\square$  —  $+dF/dt$ ,  $\triangle$  —  $-dF/dt$ , I-ischaemia, R-reperfusion, \* $P$ ,0.05, \*\* $P$ <0.01, significant differences regarding the baseline values before ischaemia. Mean  $\pm$  S.E.M. from five experiments. One way ANOVA + Newman-Kuels test.

and  $-dF/dt$  were similar during ischaemia in both experimental groups. However, after 60 min of relaxation all of measured parameters were stayed significantly lower regarding the baseline values only in group treated with 1 mM of 4-AP.

*The effects of 4-AP on the phenylephrine inotropic action in rat isolated papillary muscle subjected to ischaemia/reperfusion procedure*

As Fig. 2 shows, phenylephrine exerted a concentration-dependent positive inotropic action in the control group of preparation, but a negative one in the preparation previously subjected to ischaemia/reperfusion procedure. Addition of 1 mM of 4-AP prevented this paradoxical effects of phenylephrine, although those effects were still significantly lower regarding control phenylephrine concentration-effects curve.

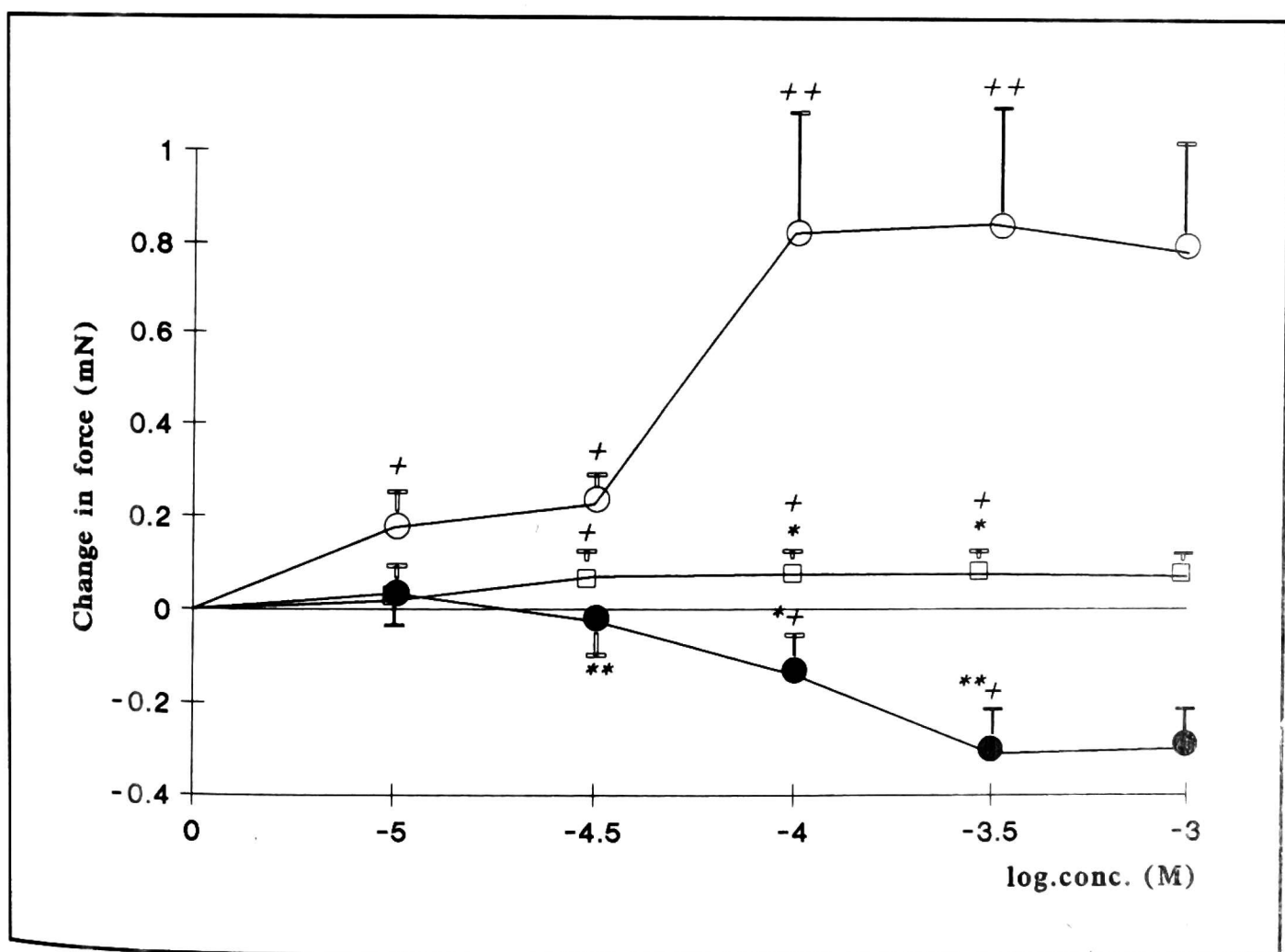


Fig. 2. The effects of phenylephrine on the force of contraction of rat papillary muscle non-treated with ischaemia (○), treated with ischaemia/reperfusion (●), and treated with ischaemia/reperfusion in the presence of 1 mM of 4-AP (□).

\* $P < 0.05$ , \*\* $P < 0.01$ , significant differences regarding the corresponding values of phenylephrine in preparations non-treated with ischaemia; +  $P < 0.05$ , ++  $P < 0.01$ , significant differences regarding the baseline, control values in each experimental groups Mean  $\pm$  S.E.M. from five experiments.

ANOVA + Newman-Kuels test.



*The effects of 4-AP on the changes in lusitropism induced by simulated ischaemia and phenylephrine*

*Table 1.* presents data concerning ttp/tt<sub>10</sub> ratio changes after 45 min of simulated ischaemia and 60 min of reperfusion in the presence and in the absence of 1 mM of 4-AP. Additionally, the significant changes in this ratio caused by phenylephrine before ischaemia as well as after ischaemia/reperfusion in preparations pretreated with 4-AP or without this intervention are presented in *Table 2*. It can be seen that 4-AP prevented a strong negative lusitropic effect observed in the control group of preparations after 45 min of ischaemia. Phenylephrine itself has shown the positive lusitropic action at lower concentrations and the negative lusitropic effect at higher concentrations, a lack of significant lusitropic action after simulated ischaemia/reperfusion and the negative lusitropic action in the preparations subjected to ischaemia but in the presence of 1 mM of 4-AP (*Table 2*).

*Table 1.* The effects of 4-aminopyridine (4-AP) on the ttp/tt<sub>10</sub> ratio during ischemia and reperfusion in rat isolated papillary muscle

	Control	After 45 min of ischaemia	After 60 min of reperfusion
In absence of 4-AP	0.94 ± 0.04	0.65 ± 0.07 **	0.81 ± 0.04
In the presence of 1 mM of 4-AP	0.80 ± 0.04	0.90 ± 0.10	0.94 ± 0.03

\*\* P < 0.01; significant regarding control values. Mean ± SEM, n = 5. ANOVA + Newman-Keuls test.

*Table 2.* The effects of phenylephrine on the ttp/tt<sub>10</sub> ratio of rat isolated papillary muscle non-treated with ischaemia, treated with ischaemia/reperfusion (I/R), and treated with ischaemia in the presence of 1 nM of 4-AP.

	Control	30 µM	300 µM
Phenylephrine	0.90 ± 0.03	0.96 ± 0.03 *	0.82 ± 0.04 *
After I/R	0.84 ± 0.05	0.80 ± 0.03	0.87 ± 0.03
After I/R in the presence of 4-AP	0.91 ± 0.03	0.88 ± 0.03	0.77 ± 0.04 *

\* P < 0.05, significant differences regarding the control values; ANOVA + Newman-Kuels test. Mean ± SEM from five experiments.

## DISCUSSION

The main finding of this paper is that 4-AP increases a depression of force of contraction and velocity of contraction induced by simulated ischaemia, but improves velocity of relaxation and prevents strong negative lusitropic effect of ischaemia. Moreover, the negative inotropic action of phenylephrine observed in stunned rat isolated left papillary muscle at 100 and 300  $\mu\text{M}$  was reversed into slight positive inotropic action in preparation underwent simulated ischaemia/reperfusion in the presence of 1 mM of 4-AP. Literature data indicate that the main electrophysiological events occurring during ischaemia are activation of ATP-sensitive  $\text{K}^+$  channels ( $\text{K}_{\text{ATP}}$ ) and inwardly rectifying  $\text{K}^+$  channels ( $\text{I}_{\text{K1}}$ ) (7). The role of transient outward  $\text{K}^+$  channels in ischaemia-induced changes in electrophysiological and mechanical characteristic of heart muscle is poorly known. Potassium current which is sensitive to 4-AP is known as  $\text{I}_{\text{to1}}$ , which is calcium independent, has rapid activation and inactivation and it is  $\text{K}^+$  selective. In the rat hearts this current is dominant in ventricular myocytes (8). However, literature data indicate that at 1 mM, 4-AP can block  $\text{I}_{\text{Kur}}$  and  $\text{I}_{\text{sus}}$ , subtypes of ultra-rapid delayed rectifier  $\text{K}^+$  channels (4). There are two main points in this paper remaining unexplained. First, what is the reason for augmentation of a sensitivity of rat isolated cardiac muscle to simulated ischaemia in the presence of 1 mM of 4-AP; and second, a protective effect of 4-AP addition during ischaemia on the negative inotropic action of phenylephrine which occurred in preparations subjected to ischaemia and non-treated with 4-AP.

Among many different mechanisms involved in heart adaptation to ischaemia, activation of  $\text{I}_{\text{K(ATP)}}$  current is one of the main event (9, 10, 11). It is in accordance with results previously obtained in our laboratory that glibenclamide strongly reversed a depression of rat heart contractility by simulated ischaemia and prevented the negative inotropic action of phenylephrine in rat stunned myocardium. Terikalant, a specific blocker of inward rectifying  $\text{K}^+$  current at 1  $\mu\text{M}$  has shown similar, but not so strong effects as glibenclamide (2). The results presented here demonstrated that 4-AP exerted the significant positive inotropic action in rat isolated papillary muscle. Since inhibition of potassium outward currents leads to the prolongation of action potential duration, consequently accompanied by enhancement in calcium influx, the positive inotropic action is understandable (12). Moreover, such a positive inotropic intervention causes a faster and deeper energetical depletion of heart muscle during ischaemia. Hence an increase in sensitivity to ischaemia in these preparations can be expected. Interestingly,  $\text{Fc}$  and  $+\text{dF}/\text{dt}$ , but not  $-\text{dF}/\text{dt}$  were more depressed after ischaemia/reperfusion period in the presence of 4-AP. Moreover, a positive lusitropic action of 4-AP during ischaemia was noted. It means that calcium-overload was reduced in the

presence of 4-AP. One of explanation for this phenomenon can be a stronger activation of uninhibited  $I_{K(ATP)}$  and  $I_{K1}$  under such a condition. Moreover, other mechanisms involved in contractile failure of heart muscle during ischaemia and the influence of 4-AP should be considered (13, 14, 15). However, prevention of negative inotropic action of phenylephrine under such a condition awaits explanation. In light of previously reported data, it seems that improving of a resistency of heart muscle to ischaemia (regarding its contractility) (2) lead to the prevention of negative inotropic action of phenylephrine. In this paper, however, it has been shown that pretreatment with 4-AP, despite of an increase in sensitivity of the rat isolated heart muscle to ischaemia also prevents the phenylephrine induced negative inotropic action. It is noteworthy that phenylephrine induced negative inotropic action in rat cardiac tissue previously subjected to ischaemia/reperfusion was chloroethylclonidine (CEC)-sensitive, but not WB 4101 sensitive (1). This suggests an involvement of  $\alpha_{1b}$  adrenergic receptors in above mentioned action of phenylephrine, since CEC is known as irreversible blocker of this subtype of receptor. There is evidence that stimulation of this subtype of adrenergic receptor activates 4-AP sensitive  $K^+$  channels in rat heart (16). Thus, it could explain a lack of significant negative inotropic action of phenylephrine in rat cardiac preparation subjected to ischaemia in the presence of 4-AP. However, as *Fig. 2* shows, the positive inotropic action of phenylephrine was also reduced under such a condition. It could mean that 4-AP interferes with positive inotropic action of phenylephrine or that stimulation of  $\alpha_{1b}$ -adrenoceptor influences some other mechanisms in cardiomyocytes, as inhibition of adenylyl cyclase (17), or activation of ATP-sensitive  $K^+$  channels (2).

To conclude, the results presented in this study demonstrate that inhibition of outward potassium current sensitive to 4-AP, enhances suppression of force and velocity of contraction but improves lusitropic ability of rat heart muscle during ischaemia and prevents the negative inotropic action of phenylephrine in rat heart muscle after ischaemia/reperfusion period.

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Author's address: Department of Cardiovascular Disease, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan  
 fax: 81-3-5684-6295; E-mail: ivo.card@mri.tmd.ac.jp