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The influence of prostacyclin (PGI_2) on contractile properties of isolated right ventricle of rat heart

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Abstract. The mechanism of the positive inotropic effect of prostacyclin (PGI_2) (2.6×10^{-6} mol/l) on the isolated right ventricle of rat heart was studied. Our results show that the positive inotropic effect of prostacyclin is produced indirectly through beta adrenoceptors and slow Ca^{2+} channels, because blockade of slow Ca^{2+} channels with verapamil (10^{-6} mol/l) and beta adrenoceptors with propranolol (10^{-6} mol/l) abolishes this effect. Alpha adrenoceptors do not mediate the action of PGI_2.
Key words. Prostacyclin; propranolol; verapamil; right ventricle; inotropic effect.

Prostacyclin (PGI_2), the cyclooxygenase product of arachidonic acid, exerts many biological effects[1]. Because of its "cytoprotective" and other beneficial effects PGI_2 is used in the therapy of various cardiovascular diseases[2–5]. The effect of PGI_2 and other related compounds (PGA, $PGA_{2\alpha}$, PGE_1, $PGF_{2\alpha}$) on heart function has been extensively studied. Some authors showed that PGI_2 and other related compounds produce inotropic effects on isolated heart and myocardial cells[6–11]. We have also studied this action of PGI_2 and we found that it produces a positive inotropic effect on the isolated right ventricle of rat heart, followed by a fall in the myocardial content of glycogen[12,13]. But, in spite of intensive experimental study, the mechanism of inotropic effect of PGI_2 and other prostaglandins is still obscure and the suggestions of some authors are contradictory[14,15]. As it is known that Ca^{2+} ions and both alpha and beta adrenoceptors are important for the mechanism of myocardial contractility, we examined the inotropic effect of PGI_2 under the blockade of slow Ca^{2+} influx with verapamil, blockade of alpha adrenoceptors with prazosin, and blockade of beta adrenoceptors with propranolol.

Materials and methods

Wistar rats weighting 200–210 g were used in these experiments. All experimental animals were divided between twelve experimental groups of seven animal each. After killing the heart was rapidly removed and the right ventricle was dissected off. Each ventricle was put on a boot electrode which was then placed in a bath of Tyrode's solution of the following composition (in mmol): NaCl 136.9, KCl 2.69, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42 and glucose 5.55, bubbled with 95% O_2/5% CO_2. The volume of the bath was 25 ml, pH = 7.4 and its temperature kept at 37 °C. Contractions were induced with square wave impulses of twice the diastolic treshold and a duration of 5 ms at a frequency of 1 Hz. The contractions were recorded via an isometric transducer (sensitivity of 0.05 g/cm) into a microdynamometer (7050, Ugo Basile). The preparation was stabilized for 30 min and after that prostacyclin (Flolan, Wellcome, UK) at a dose of 2.6×10^{-6} mol/l, which produces a maximal positive inotropic effect, was added to the bath and contractions were recorded continuously for another 30 min. For investigation of the possible prostacyclin mechanism of action prazosin (Sigma, USA) 10^{-6} mol/l, propranolol (ICI, Manchesfield, UK) 10^{-6} mol/l and verapamil (Zdravlje, Yugoslavia) 10^{-6} mol/l were used. They were added to the bath at the same time as prostacyclin or 5 or 15 minutes before it. As a control we used a group of 7 animals whose ventricles were subjected to the identical experimental procedure without investigated substances. In these preparations the amplitude of contractions did not change during the whole time of experiment (30 min). Results were presented (see figures) as percentage change from the starting point. Statistical analysis was performed by Student's t-test for paired observations. The means $\pm$SEM of n observations are quoted in the text and figures, and $p < 0.05$ was considered statistically significant.

Results

First we investigated the influence of prostacyclin on the inotropic effect of isolated rat right ventricle. We found a dose-dependent positive inotropic effect with doses of 2.6×10^{-6} to 1×10^{-5} mol/l. A higher concentration of prostacyclin (2.6×10^{-5} mol/l) had a negative inotropic effect (fig. 1).

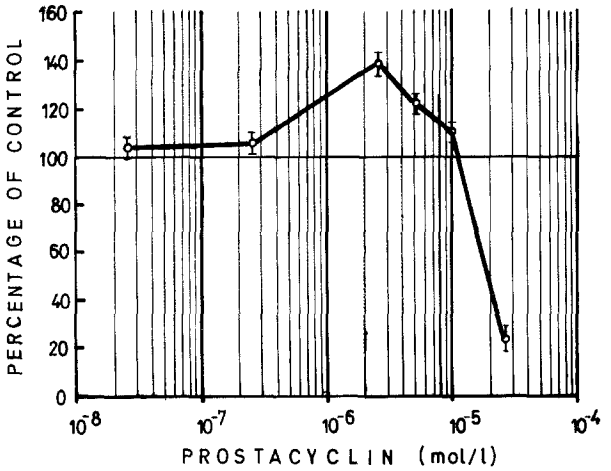

Figure 1. Effects of prostacyclin on the amplitude of contraction of isolated right ventricle of rat heart. Points (mean ±SEM, n = 7) are maximum percentage of changes from values immediately preceding prostacyclin administration.

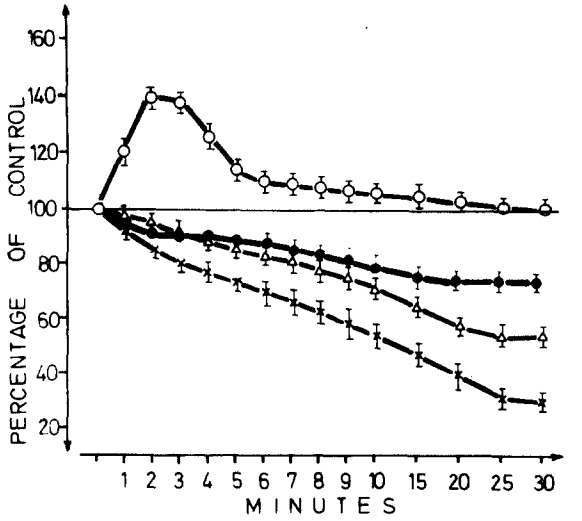

Figure 2. Effects of prostacyclin (2.6×10^{-6} mol) (○), prazosin (10^{-6} mol) (●), propranolol (10^{-6} mol) (△) and verapamil (10^{-6} mol) (×) on the amplitude of contraction in isolated right ventricle of rat heart, expressed as percentage of control. Each point represents mean ±SEM (n = 7).

Figure 2 shows the results of experiments in which prostacyclin, prazosin, propranolol and verapamil were used separately. PGI_2 at a concentration of 2.6×10^{-6} mol/l induced a maximal positive inotropic effect on the isolated right ventricle of rat heart when electrically stimulated. The effect of prostacyclin was manifested immediately after its addition to the bath, and the maximal effect was attained at the end of the 2nd minute when the amplitude of contraction increased 139.47%. This increase in the amplitude of contraction is statistically significant ($p < 0.001$). After that the amplitude of contraction of the ventricle returned to the initial, control value before the end of experiment (30 min). On the other hand, immediately after addi-

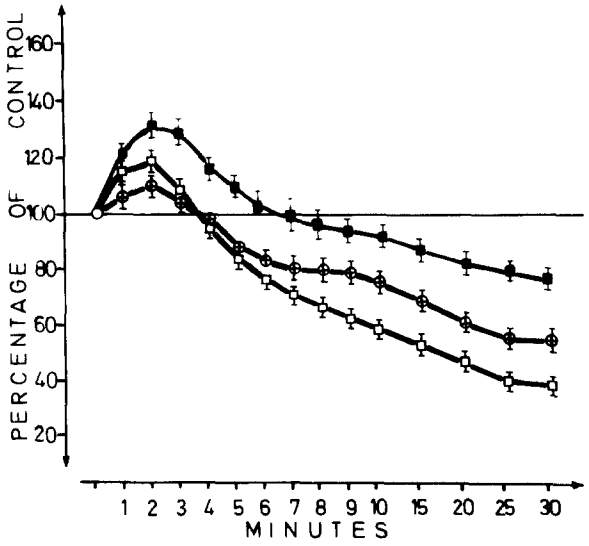

Figure 3. Effects of simultaneous action of prostacyclin with prazosin (■), propranolol (⊕) or verapamil (□) on the amplitude of contraction in isolated right ventricle of rat heart, expressed as percent of control. Each point represents mean ± SEM (n = 7).

tion of prazosin at a concentration of 10^{-6} mol/l, propranolol at 10^{-6} mol/l and verapamil at 10^{-6} mol/l, the amplitude of contraction of the right ventricle was decreased as the result of the inhibitory action of these substances. We obtained maximal inhibition after about 25 min. The amplitude decreased with prazosin to 70.58%, with propranolol to 54.50% and with verapamil to 20.77%, and no further decline was observed.

When prostacyclin was used simultaneously with prazosin, propranolol or verapamil the positive inotropic effect was also seen during the first 2 min but the effect was less than when prostacyclin was used alone (PGI_2 + prazosin 130.25%, PGI_2 + propranolol 109.09% and PGI_2 + verapamil 119.23%). In all cases the amplitude of contraction then declined continuously until the 25th minute of experiment (fig. 3).

Figure 4 shows the effect of PGI_2 after 5 min of prazosin, propranolol or verapamil action. The positive inotropic effect of PGI_2 on the isolated right ventricle of the rat heart was completely abolished by propranolol. After treatment with verapamil PGI_2 produced only a slight insignificant increase in the amplitude of contraction in the 6th minute, while after prazosin treatment the positive inotropic effect of prostacyclin was nearly the same as in experiments where prostacyclin was used alone. It reached 117% because the starting point was 13% below the control value.

The positive inotropic effect of PGI_2 after 15 min of verapamil action was completely abolished, while after 15 min of prazosin action PGI_2 still caused a positive inotropic effect (fig. 5).

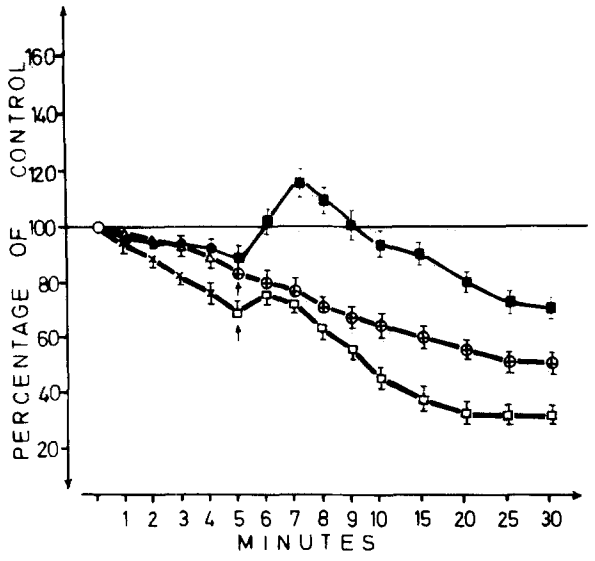

Figure 4. Prostacyclin (↑) was added after 5 minutes of prazosin (■), propranolol (⊕) or verapamil (□). Effect of prostacyclin was completely abolished with propranolol. There was a slight insignificant increase in amplitude of contraction with verapamil while prazosin did not inhibit the positive inotropic effect of prostacyclin. Each point represents mean ±SEM (n = 7).

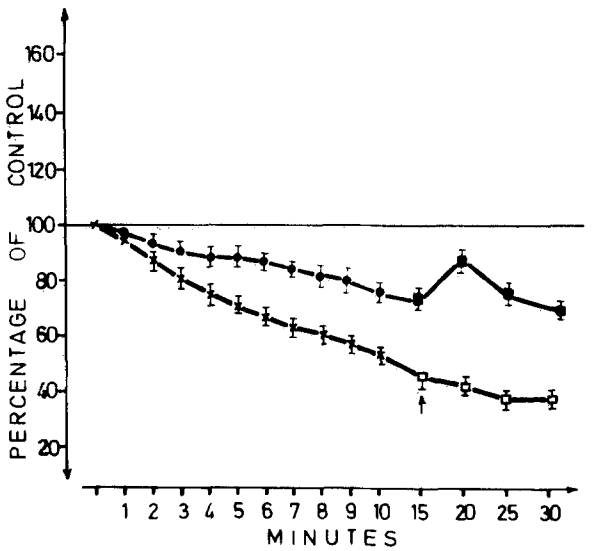

Figure 5. Prostacyclin was added (↑) after 15 minutes of prazosin (■) or verapamil (□) action. Verapamil abolished, while prazosin did not inhibit the positive inotropic effect. Each point represents mean ±SEM (n = 7).

Discussion

The effect of PGI_2 on the contractile properties of myocardium has been extensively studied. Some authors found that PGI_2 exerts a dose-dependent positive inotropic effect on isolated guinea-pig or rat heart[7,11,14], isolated rat or guinea-pig atria[16,17], or isolated rat or guinea-pig myocardial cells[18,19]. On the other hand, some authors found that PGI_2 did no exert any effect on contracile force[15,20,21] or produced only a negative inotropic effect on rat heart cells[9]. We also found that prostacyclin exerts a dose-dependent positive inotropic effect on the isolated right ventricle of rat heart. This effect is of short duration[12] and is probably due to rapid degradation of PGI_2[19]. It is known that PGI_2 is an unstable compound with $t_{1/2}$ of 10 minutes in aqueous solution at 37 °C[22], when it is converted to the stable product 6-keto-$PGF_{1\alpha}$ which does not affect contractile force[19]. The mechanism by which PGI_2 produces a positive inotropic effect is under discussion. Many authors working on the isolated heart and myocardial cells from rat and guinea-pig found that PGI_2 modifies transmembrane calcium ion movement[11,18,19,23]. In the isolated guinea-pig heart it was found that PGI_2 may promote Ca^{2+} entry through slow channels[22]. On the other hand, the positive inotropic-induced effect of PGI_2 was attributed to norepinephrine release which stimulated contraction[8]. It was shown that PGI_2 is a potent stimulator of adenylate cyclase in several cell types[24], and a significant increase in cAMP content was also found to accompany the enhancement in contractile force in isolated rat atrium[16]. The pharmacological and physiological action of PGI_2 can be explained through adenylate cyclase stimulation and consequent activation of protein kinase A by cAMP and augmentation of calcium channel activity[19].

In our experiments we tried to elucidate the mechanism of the prostacyclin effect by the use of prazosin (alpha-adrenoceptor blocker), propranolol (beta-adrenoceptor blocker) and verapamil (Ca^{2+}-slow channel blocker). The results obtained show that after prazosin action the amplitude of contraction was nearly the same as in experiments where prostacyclin was used alone. It does not reach this value because the amplitude of contraction was already lowered as a result of inhibitory action of prazosin. According to our results, alpha adrenoceptors are not involved in mediating the PGI_2 action. The other two blockers (propranolol and verapamil) modify the positive inotropic effect of prostacyclin. Propranolol requires only 5 min to block beta adrenoceptors while verapamil requires 15 min since more time is required for blocking Ca^{2+}-slow channels, but both abolish the positive inotropic effect of prostacyclin completely. The blockade of the positive inotropic action of PGI_2 by propranolol and verapamil shows that this effect is indirect and is mediated by a mechanism involving activation of beta adrenoceptors and slow Ca^{2+} channels.

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