Differential effects of OPC-18790, amrinone and dobutamine on cardiac function and energy metabolism in the guinea-pig isolated ischaemic heart

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1 The effects of OPC-18790, a novel positive inotropic agent, on cardiac function and myocardial energy metabolism in the guinea-pig isolated heart with ischaemia were studied by ^{31}P -magnetic resonance spectroscopy (MRS) and compared with those of amrinone and dobutamine.

2 Cardiac ischaemia was induced by intracoronary infusion of 15 μm microspheres to reduce coronary perfusion flow (CPF) by 50%. Microsphere embolisation caused a 40% decrease in left ventricular systolic pressure (LVSP), cardiac contractility measured by peak of ventricular pressure development (LVdP/dt) and slightly reduced heart rate. There was also a decrease in ATP and creatine phosphate (PCr) by 20%, an increase in inorganic phosphate (P_i) by 25% and an acidic shift of intracellular pH in the ischaemic heart.

3 In the ischaemic heart, OPC-18790, amrinone and dobutamine were applied at concentrations which increased LVdP/dt by about 60%. These compounds increased LVP by 15% to 30% and increased CPF by about 10%. Amrinone and dobutamine but not OPC-18790 increased heart rate. When these drugs produced the haemodynamic changes described above, amrinone and dobutamine reduced ATP and PCr, increased P_i and produced further intracellular acidosis, whereas, OPC-18790 did not change these parameters.

4 Cardiac pacing at 285 beats min^{-1} produced decreases in LVP, LVdP/dt and CPF by about 30%, 20%, 5%, respectively and an increase in P_i , decreases in PCr and ATP, and intracellular acidosis.

5 These results suggest that degradation of high energy phosphate compounds closely relates to increase in heart rate in the ischaemic heart. Positive inotropic agents without chronotropic action seem to be beneficial in support of the ischaemic heart.

Keywords: ^{31}P -magnetic resonance spectrometry; high energy phosphates; Langendorff's preparations; ischaemic heart; positive inotropic agents; OPC-18790; amrinone; dobutamine

Introduction

Myocardial energetics and cardiac function are closely related because the heart is a highly anaerobic organ. Cardiac ischaemia produces cardiac dysfunction, however, positive inotropic intervention in heart failure originating from coronary heart disease or cardiac ischaemia is still controversial (Katz, 1986; 1988). An increase in contractile force requires a greater myocardial oxygen supply and many inotropic agents have chronotropic and vasodilator or vasoconstrictor actions as well as positive inotropic actions. Therefore, the usefulness of these drugs in the ischaemic heart is determined by the balance of these actions. In fact, deleterious effects of positive inotropic stimulation in ischaemic hearts have been reported (Rude *et al.*, 1980; Pozen *et al.*, 1981).

Catecholamines such as dobutamine and dopamine are the most popular positive inotropic agents in the clinical stage, but the usefulness of catecholamines is limited by their positive chronotropic and arrhythmogenic actions. In the last two decades, many efforts have been made to develop new types of positive inotropic agents. Of these new positive inotropic agents (Taira, 1987), many are guanosine 3':5'-cyclic monophosphate (cyclic GMP)-inhibited phosphodiesterase (cGI-PDE) inhibitors such as amrinone (Alousi *et al.*, 1979). Recently, we described a new compound, OPC-18790 [($\pm$)-6-[3-(3,4-dimethoxybenzylamino)-2-hydroxypropoxy]-2-(1*H*)-quinolinone] (Fujioka *et al.*, 1992) which is a

positive inotropic agent with a moderate coronary vasodilator action, lacking a direct chronotropic action (Hosokawa *et al.*, 1992). The cardiovascular profile of OPC-18790 is different from that of the pure cGI-PDE inhibitor, amrinone (Sato *et al.*, 1986) and other cGI-PDE inhibitors (Taira, 1987). It has been shown that the mechanism of positive inotropic action of OPC-18790 involves prolongation of the duration of the action potential in ventricular myocytes as well as inhibition of cGI-PDE (Hosokawa *et al.*, 1992). Lack of direct chronotropic action and weaker vasodilator action compared to positive inotropic action are thought to be beneficial in inotropic therapy for the ischaemic heart.

Recent progress of ^{31}P magnetic resonance spectroscopy (^{31}P -MRS) enables simultaneous measurement of cardiac functions and cardiac energetics. In this study, we used the ^{31}P -MRS to evaluate the effects of OPC-18790 on energy metabolism during positive inotropic treatment in the guinea-pig isolated ischaemic heart in comparison with amrinone and dobutamine.

Methods

Isolated heart preparations

The experiment was conducted on guinea-pig isolated heart preparations. Male guinea-pigs (Hartley strain, SLC, Shizuoka, Japan) weighing 300–400 g were anaesthetized with pentobarbitone (50 mg kg^{-1} , i.p.). The chest was opened

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and the heart immediately isolated. A catheter was inserted retrogradely from the aorta, and the heart was perfused by Langendorff's method at a constant pressure of 80 cmH₂O. The perfusate used was modified Krebs-Henseleit solution (composition in mM: NaCl 119, CaCl₂ 2.5, KCl 4.8, MgSO₄ 1.2, KH₂PO₄ 0.12, NaHCO₃ 24.9, glucose 10) bubbled with a gas mixture of 95% O₂ and 5% CO₂, and maintained at 37°C.

Magnetic resonance spectroscopy

The heart preparation was placed in a magnetic resonance probe (20 mm in diameter). ³¹P-magnetic resonance spectra (³¹P-MRS) were obtained using a magnetic resonance spectrometer Fx 200 (JEOL, Tokyo, Japan) operating at 80.76 MHz in the Fourier transform mode. For each spectrum 100 free induction decays (FIDs) were acquired with a 5 kHz spectral width. Repetition time was 3 s. Data point for the FID acquisition was 1024, and zero-filled to 4096 before Fourier transformation. Tissue levels of ATP, creatine phosphate (PCr) and inorganic phosphate (P_i) were estimated by integrating areas under individual peaks. The intracellular pH was estimated from the chemical shift (S) of P_i peak from PCr peak by the following equation postulated by Flaharty *et al.* (1982); $\text{pH} = 6.90 - \log [(S-5.85)/(3.29-S)]$

Cardiac function

A Latex balloon was introduced into the left ventricle chamber from the left atrium appendage to measure left ventricular pressure (LVP). The first derivative (LVdP/dt) was determined from the LVP signals with electronic differentiator (Polygraph EQ-45764, Nihon Kohden, Tokyo, Japan) and used as an index of cardiac contractility. Heart

rate was determined with a cardiometer (AF601G, Nihon Kohden) triggered by the LVP signals. Coronary perfusion flow (CPF) was determined by a volume of effluent measured every min. Recordings were made on charts with a thermal pen recorder (Recticorder, Nihon Kohden).

Production of cardiac ischaemia

After the haemodynamics of the heart preparation became stable, patchy ischaemia was produced by injecting microspheres (15 µm in diameter) into the coronary artery until the CPF decreased to 50% of the basal level ($4-8 \times 10^6$ beads). After the haemodynamic changes due to ischaemia had stabilized, OPC-18790, amrinone or dobutamine was continuously infused into the coronary artery at an infusion rate of 1% of CPF for 15 min with an infusion pump. Each preparation was treated with one of the test compounds.

Cardiac pacing

Cardiac pacing was performed in another six preparations after patchy ischaemia was produced by injecting microspheres. A pacing electrode was placed on the right atrium and the hearts were electrically driven by a rectangular pulse wave (duration 3.0 ms and intensity 120% of threshold) using an electronic stimulator (SEN-3201, Nihon Kohden) to maintain a rate of 285 beats min⁻¹.

Drugs

The test compounds used were OPC-18790 (Lot. 2D94M, Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan), amrinone (Lot. 921106, synthesized in Otsuka Pharmaceutical Co. Ltd.) and dobutamine HCl (Lot. 1001, Shionogi Phar-

Figure 1 Representative traces of ischaemic changes in cardiac functions and energy metabolism in Langendorff preparations of guinea-pig heart. The traces show left ventricular pressure (LVP), the first derivative of the LVP (LVdP/dt) and heart rate (HR), and ³¹P-magnetic resonance spectra illustrate the inorganic phosphate (P_i), creatine phosphate (PCr) and adenosine triphosphate (ATP). The horizontal bar represents 1 min.

maceutical Co., Osaka, Japan). OPC-18790, amrinone and dobutamine were dissolved in 1.5 mM lactate containing modified Krebs-Henseleit solution at a concentration of 1, 20 and 0.03 mM, respectively.

Statistics

Values are expressed as mean $\pm$ s.e.mean. The difference between mean values before and after ischaemia was analysed by Student's paired *t* test. Differences of drug effects or basal values of each groups after ischaemia were analysed by non-parametric 1 way ANOVA then multiple comparison with Tukey's method using SAS. When $P < 0.05$, differences were considered significant.

Results

Energetic and functional changes produced by cardiac ischaemia

Coronary perfusion flow (CPF), left ventricular systolic pressure (LVSP), the first derivative of the left ventricular pressure (LVdP/dt), heart rate (HR), and the double product (DP) between the LVP and HR were measured after stabilization. Basal values in the normal heart were 13.7–14.8 ml min⁻¹, 88.7–94.5 mmHg, 1190–1330 mmHg s⁻¹, 217–242 beats min⁻¹ and 19230–23030 mmHg beats min⁻¹, respectively ($n = 24$). Injection of microspheres produced cardiac ischaemia with decreases in LVP, CPF, LVdP/dt and DP (typical traces are shown in Figure 1a). There were also decreases in the PCr and ATP signals and an increase in the P_i signal with the chemical shift moved upward, indicating intracellular acidosis (typical spectrograms are shown Figure 1b). Ischaemia caused LVP, CPF, LVdP/dt, HR and DP by 60.8%, 50.4%, 55.1%, 94.2% and 56.5% of their respective basal values ($n = 24$, Figure 2a). In addition adenosine triphosphate (ATP) and creatine phosphate (PCr) decreased to 85.7% and 92.5% of their respective basal values whilst inorganic phosphate (P_i) increased to 123.6% (Figure 2b) in the ischaemic heart. The intracellular pH showed the development of acidosis (7.16 in the normal heart and 7.04 in the ischaemic heart).

Energetic and functional changes by positive inotropic intervention with OPC-18790, amrinone and dobutamine in the ischaemic hearts

After the induction of cardiac ischaemia, these 24 hearts were randomly divided into four groups, and OPC-18790, amrinone, dobutamine or vehicle injected into the perfusion circuit. As shown in Table 1 and Figure 2, basal values in the various groups prior to drug treatment were not statistically different from each other. Typical traces of effects on cardiac functions of OPC-18790, amrinone and dobutamine are

shown in Figure 3. As shown in Figure 4, with almost equal increases in LVdP/dt, an index of cardiac contractility, OPC-18790, amrinone and dobutamine increased CPF by $13.6 \pm 3.3\%$, $12.4 \pm 1.2\%$ and $10.6 \pm 2.2\%$, respectively from the basal values after ischaemia (Figure 4a). The concentrations of OPC-18790, amrinone and dobutamine in the per-

Figure 2 Ischaemic changes in cardiac functions and parameters of energy metabolism in Langendorff preparations of guinea-pig heart. The peak of the first derivative of the LVP (LVdP/dt), left ventricular systolic pressure (LVSP), heart rate (HR), coronary perfusion flow (CPF), double product (DP), inorganic phosphate (P_i), creatine phosphate (PCr) and adenosine triphosphate (ATP) and intracellular pH (pH) levels are shown before (open columns) and after (hatched columns) ischaemia induced by microsphere embolisation. Each column represents mean $\pm$ s.e.mean of 24 preparations. * $P < 0.05$, ** $P < 0.01$ compared with values before ischaemia. Unit for DP is mmHg beats min⁻¹ $\times 10^2$ and unit for pH is log unit.

Table 1 Basal values of cardiac function and energy metabolism in the Langendorff preparation of guinea-pig ischaemic hearts

		OPC-18790	Vehicle	Dobutamine	Amrinone
LVSP	(mmHg)	55.5 $\pm$ 7.3	57.8 $\pm$ 3.6	59.7 $\pm$ 5.4	52.0 $\pm$ 2.5
LV dP/dt	(mmHg s ⁻¹)	680 $\pm$ 130	680 $\pm$ 60	750 $\pm$ 100	630 $\pm$ 40
HR	(beats min ⁻¹)	218 $\pm$ 5.2	220 $\pm$ 8.9	225 $\pm$ 5.1	209 $\pm$ 14.9
CPF	(ml min ⁻¹)	7.50 $\pm$ 0.27	6.65 $\pm$ 0.37	7.60 $\pm$ 1.0	7.48 $\pm$ 0.50
DP	(unit)	120.3 $\pm$ 15.2	126.9 $\pm$ 8.6	133.4 $\pm$ 10.1	107.7 $\pm$ 7.3
P _i	(%)	25.4 $\pm$ 8.6	23.2 $\pm$ 6.4	17.8 $\pm$ 2.8	28.0 $\pm$ 6.1
PCr	(%)	-20.9 $\pm$ 3.5	-16.1 $\pm$ 3.8	-14.6 $\pm$ 0.9	-18.2 $\pm$ 2.6
ATP	(%)	-14.3 $\pm$ 5.6	-17.4 $\pm$ 3.8	-9.9 $\pm$ 3.1	-15.5 $\pm$ 1.4
pH	(unit)	7.00 $\pm$ 0.02	7.06 $\pm$ 0.03	7.02 $\pm$ 0.03	7.08 $\pm$ 0.02

The values shown are those prior to drug treatment. LVSP: left ventricular systolic pressure, LVdP/dt: peak of the first derivative of left ventricular pressure, HR: heart rate, CPF: coronary perfusion flow, DP: double product, P_i: inorganic phosphate, PCr: creatine phosphate, ATP: adenosine triphosphate, pH: intracellular pH. Unit for DP is mmHg beats min⁻¹ $\times 10^2$ and unit for pH is log unit.

fusion fluid estimated from CPF were $14.4 \pm 1.6 \mu\text{M}$ ($n = 6$), $180 \pm 14 \mu\text{M}$ ($n = 6$) and $0.42 \pm 0.04 \mu\text{M}$ ($n = 6$), respectively. The potency of positive inotropic action determined by LVdP/dt increase of dobutamine was about 34 times greater than that of OPC-18790 and that of amrinone was 12 times less potent than that of OPC-18790. At the same positive inotropic concentrations of these drugs, OPC-18790 did not exert any change in HR ($-3.0 \pm 5.9 \text{ beats min}^{-1}$) but amrinone and dobutamine increased HR by $41.5 \pm 4.3 \text{ beats min}^{-1}$ and $92.2 \pm 8.3 \text{ beats min}^{-1}$, respectively. Therefore, OPC-18790, amrinone and dobutamine increased DP by $27.9 \pm 6.7\%$, $46.4 \pm 7.7\%$ and $63.5 \pm 7.3\%$, respectively.

When OPC-18790 increased LVSP, CPF and LVdP/dt , the compound did not change the levels of P_i , PCr and ATP and slightly reversed cardiac acidosis (Figure 4b). On the other hand, amrinone and dobutamine increased P_i and decreased PCr and ATP. Intracellular pH was lowered further by amrinone and dobutamine treatment (Figure 4b). The degree of aggravation of high energy phosphate metabolism and acidosis by amrinone and dobutamine seemed to correlate with HR increase.

Energetic and functional changes by cardiac pacing in the ischaemic hearts

To clarify the relationship between changes in energetic parameters and heart rate increase, cardiac pacing at $285 \text{ beats min}^{-1}$ was performed in the other ischaemic heart preparations. The rate of $285 \text{ beats min}^{-1}$ is about 50 beats min^{-1} higher than the baseline rate. Typical traces of LVSP, LVdP/dt and HR are shown in Figure 5. The cardiac pacing produced decreases in LVSP, LVdP/dt , DP and CPF of about 30%, 20%, 20%, 5%, respectively (Figures 5 and 6). Although these parameters of cardiac function were decreased, at that time parameters of cardiac energetics were aggravated with increases in P_i and decreases in PCr, ATP and intracellular pH (Figures 5 and 6).

Discussion

In these experiments, we used the cardiac ischaemic model induced by $15 \mu\text{M}$ microsphere embolisation. As reported previously (Hosokawa *et al.*, 1986), this model is characterized by milder ischaemia than that induced by global ischaemia. Changes in high energy phosphate compounds, inorganic phosphate and changes in cardiac function were smaller in microsphere-induced ischaemia than after global ischaemia. As the aim of these experiments was to compare the effects of several positive inotropic agents on cardiac function and energetics simultaneously in ischaemic hearts, we chose the mild ischaemic model and evaluated effects by the ^{31}P -MRS method.

When OPC-18790, amrinone and dobutamine produced almost the same increase in contractility (LVdP/dt by about 60%), amrinone and dobutamine increased HR while OPC-18790 did not (Figure 4). Increases in HR induced by amrinone (Alousi *et al.*, 1979; Zannad *et al.*, 1983; Sato *et al.*, 1986) and dobutamine (Tuttle & Mills, 1975; Sonnenblick *et al.*, 1979) have been reported previously and lack of direct chronotropic effect of OPC-18790 is also consistent with a previously reported action in canine isolated right atria (Hosokawa *et al.*, 1992). From the parameters of cardiac energetics elucidated by ^{31}P -MRS signals, it is shown that amrinone and dobutamine aggravate the cardiac energetic state, namely increase P_i , decrease PCr and ATP and cause further acidification of intracellular pH, with an increase in LVdP/dt (Figure 4). In contrast to amrinone and dobutamine, OPC-18790 did not change those parameters of energy metabolism when producing the same increase in LVdP/dt (Figure 4). These results suggest that the increase in HR and/or DP produced by amrinone and dobutamine are correlated with the changes in cardiac energetics (Figure 4). Therefore, we examined the influences of cardiac pacing at $285 \text{ beats min}^{-1}$ on the cardiac function and energetics in the ischaemic hearts. The increase in HR by cardiac pacing

Figure 3 Representative traces of the effects of OPC-18790, amrinone, and dobutamine on cardiac functions and energy metabolism in Langendorff preparations of guinea-pig heart with ischaemia. The traces show the left ventricular pressure (LVP), the first derivative of the LVP (LVdP/dt) and heart rate (HR), and ^{31}P -magnetic resonance spectra illustrate the inorganic phosphate (P_i), creatine phosphate (PCr) and adenosine triphosphate (ATP). The horizontal bar represents 1 min.

Figure 4 Effects of OPC-18790, amrinone, dobutamine and their vehicle on cardiac functions and energy metabolism in Langendorff preparations of guinea-pig heart with ischaemia. Changes in the peak of the first derivative of LVP ($LVdP/dt$), left ventricular systolic pressure (LVSP), heart rate (HR), coronary perfusion flow (CPF), double product (DP), inorganic phosphate (P_i), creatine phosphate (PCr), adenosine triphosphate (ATP) and intracellular pH (pH) from base line after ischaemia are shown. Each column represents mean $\pm$ s.e.mean of 6 preparations. * $P < 0.05$, ** $P < 0.01$ compared with values before drug treatment. † $P < 0.05$, †† $P < 0.01$ compared with response of OPC-18790. ‡ $P < 0.05$, ‡‡ $P < 0.01$.

Figure 5 Representative traces of the effects of cardiac pacing at 285 beats min⁻¹ on cardiac functions and energy metabolism in Langendorff preparations of guinea-pig heart with ischaemia. The traces show the left ventricular pressure (LVP), the first derivative of the LVP ($LVdP/dt$) and heart rate (HR), and ³¹P-magnetic resonance spectra illustrate the inorganic phosphate (P_i), creatine phosphate (PCr) and adenosine triphosphate (ATP). The horizontal bar represents 1 min.

Figure 6 Effects of cardiac pacing at 285 beats min^{-1} on cardiac functions and energy metabolism in Langendorff preparations of guinea-pig heart with ischaemia. Changes in the left ventricular systolic pressure (LVSP), the peak of the first derivative of the left ventricular pressure (LVdP/dt), coronary perfusion flow (CPF), double product (DP), inorganic phosphate (P_i), creatine phosphate (PCr), adenosine triphosphate (ATP) and intracellular pH (pH) after rapid atrial pacing are shown. Each column represents mean $\pm$ s.e.mean of 6 preparations.

aggravates cardiac energetics and decreases cardiac functions such as LVSP, LVdP/dt and DP (Figure 5). From these results, energetic aggravation seems to correlate closely with an increase in HR rather than DP in the ischaemic heart model.

Furthermore, OPC-18790 (Hosokawa *et al.*, 1992) and amrinone (Sato *et al.*, 1986) have direct coronary vasodilator actions, but dobutamine has a vasoconstrictor action via an α -adrenoceptor partial agonist effect (Vatner *et al.*, 1974; Kenakin, 1981; Ruffolo *et al.*, 1981) as well as a β -adrenoceptor agonist action. Dobutamine produced a weaker increase in CPF in proportion to a large increase in DP and this also contributed to the aggravation of cardiac energetics by dobutamine (Figure 4). It is reported that amrinone has cGI-PDE inhibiting action (Endoh *et al.*, 1982; Honerjäger, 1981); the inhibition of cGI-PDE may explain positive inotropic, positive chronotropic and vasodilator actions of amrinone. It has also been demonstrated that OPC-18790 has a cGI-PDE inhibiting action (Hosokawa *et al.*, 1992) and an increase in intracellular cyclic AMP level may be involved in the inotropic effect of OPC-18790 (Endoh *et al.*, 1994). In contrast to amrinone, the positive inotropic action of OPC-18790 was exerted rather than the vasodilator action and it also has no chronotropic effect (Figure 4). Another mechanism other than inhibition of cGI-PDE, for example, potassium channel inhibition (Takase *et al.*, 1994) may be involved in the expression of the whole haemodynamic action of OPC-18790.

One of the explanations of the finding that OPC-18790 improved the cardiac function without changes in cardiac energetics is the well-balanced cardiovascular profile of OPC-18790, i.e., positive inotropic action with little chronotropic action and moderate coronary vasodilator action. Recently, we also showed that OPC-18790 improved cardiac function without changing cardiac energetics in a ^{31}P -MRS study using open-chest dog with cardiac ischaemia (Ishikawa *et al.*, unpublished data).

In conclusion, effects of positive inotropic agents on cardiac energetics are different from each other and energetic aggravation seems to correlate closely to HR increase in the ischaemic heart. A positive inotropic agent without direct chronotropic action may be beneficial in ischaemic hearts.

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