EFFECTS OF LANDIOLOL ON MECHANICAL AND METABOLIC CHANGES IN RAT REPERFUSED ISCHAEMIC HEARTS

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SUMMARY

1. The aim of the present study was to clarify the effects of landiolol, a short-acting selective β₁-adrenoceptor blocking agent, on mechanical and metabolic changes in postischaemic perfused hearts.

2. Rat isolated hearts (n = 30) were randomly separated into non-ischaemic or ischaemic groups. The latter group was further divided into Krebs’–Henseleit solution (KHS)- and landiolol (30, 100 or 300 μmol/L)-treated groups. Ischaemic hearts were subjected to 25 min global ischaemia and 20 min reperfusion under atrial pacing. Time-course changes in left ventricular (LV) end-diastolic pressure (LVEDP), LV developed pressure (LVPD), peak positive velocity of change of LV pressure (LVdP/dtₚₑₓ), and coronary flow were observed along with tissue contents of adenosine triphosphate (ATP), creatine phosphate, inorganic phosphate (Pi), malondialdehyde (MDA) and lactate dehydrogenase (LDH) release in coronary effluent. The effects of landiolol on rat isolated aortic preparations under KCl contraction were also investigated.

3. Ischaemia–reperfusion significantly impaired cardiodynamics, such as LVEDP, LVPD and LVdP/dtₚₑₓ, decreased myocardial ATP content and increased Pi and LDH release. In the 30 μmol/L landiolol-treated group, cardiovascular parameters impaired by ischaemia–reperfusion and increased LDH release were further exacerbated and myocardial MDA content was significantly increased. In the 300 μmol/L landiolol-treated group, cardiac contractile dysfunction was improved and myocardial MDA, ATP and Pi contents were preserved. All measurements in the 100 μmol/L landiolol-treated group were similar to those in the ischaemic KHS group. Furthermore, significant relaxations of isolated aortic preparations were obtained with landiolol 30–1000 μmol/L, suggesting a possible calcium antagonism with landiolol.

4. In conclusion, landiolol, at low concentrations, aggravated myocardial ischaemia–reperfusion injuries, whereas at high concentrations it ameliorated them. The former effect may be mediated by the production of reactive oxygen species, whereas the latter may involve calcium antagonist activity.

Key words: calcium antagonism, cardiodynamics, ischaemia–reperfusion, landiolol, reactive oxygen species.

INTRODUCTION

In isolated and perfused hearts, decreased oxygen supply (i.e. ischaemia) can induce the intra- and extra-cellular production of reactive oxygen species (ROS) during reperfusion and the following is advocated as the mechanism of ROS production: xanthine dehydrogenase in the myocardium is converted into xanthine oxidase in the presence of intracellular Ca²⁺, and this xanthine oxidase produces ROS, such as superoxide and H₂O₂, by using xanthine and hypoxanthine as substrates. In ischaemic myocardium, intracellular Ca²⁺ is increased and so xanthine oxidase production is enhanced. Moreover, the increase in intracellular Ca²⁺ levels leads to degradation of ATP in myocardial cells. Because ATP is broken down into xanthine and hypoxanthine, it is considered that decreases in intracellular ATP correspond to increases in the substrates for xanthine oxidase. Thus, whatever a large amount of oxygen is supplied during reperfusion, a lot of ROS are produced in the myocardium where xanthine oxidase and its substrates are increased by ischaemia. Increased ROS can induce ultrastructural alterations and increase membrane phospholipids and/or mitochondrial defective function in the myocardium. These events are involved with ischaemia–reperfusion injuries, such as reperfusion arrhythmias, cardiac dysfunction, myocardial stunning and cell death. Therefore, measurements of myocardial ATP content, intracellular Ca²⁺ levels and lipid peroxidation would be useful for elucidation of mechanisms of ischaemia–reperfusion injury.

Llandiolol, which was used in the present study, is a short-acting selective β₁-adrenoceptor antagonist. This drug does not show a membrane stabilizing action and intrinsic sympathomimetic activity. Compared with esmolol, another short-acting selective β₁-adrenoceptor antagonist, landiolol has higher cardioselectivity and more potent β-adrenoceptor blocking activity. In the postischaemic perfused heart, esmolol showed a dose-dependent cardioprotective effect and decreased lipid peroxidation, which suggests an antioxidant effect. Conversely, landiolol has been reported to enhance postischaemic cardiac function after warm cardioplegic arrest in rat isolated hearts. Furthermore, the possibility of cardioprotective effects of landiolol against ischaemia–reperfusion injuries was shown in guinea-pig isolated hearts. However, in those studies, it was not confirmed what action of landiolol contributed to its cardioprotective effect.
Thus, the aim of the present study was to clarify the effects of landiolol on mechanical and metabolic changes in postischaemic perfused hearts. Therefore, in the present experiments, cardiac function was observed and tissue contents of high-energy metabolites and malondialdehyde (MDA), as an index of lipid peroxidation, were measured in addition to observation of the effects of landiolol on isolated aortic preparations.

METHODS

Animals used in the present study were handled in accordance with Guidelines for Animal Experimentation of University of the Ryukyus and experimental protocols were approved by the Animal Care and Use Committee of this institution.

Experimental preparation

Male Wistar rats (SLC, Shizuoka, Japan), weighing 230–290 g, were anaesthetized with diethylether and sodium heparin (1000 IU/kg) was injected intravenously. After thoracotomy, the heart was excised rapidly and cannulated retrogradely via the aorta on a Langendorff apparatus. The isolated heart was perfused at a constant pressure of 100 cm H2O with 37°C Krebs Henseleit solution (KHS) of the following composition (in mmol/L): NaCl 120; KCl 4.80; CaCl2 1.25; MgSO4 1.20; KH2PO4 1.20; NaHCO3 25.0; glucose 11.0. The perfusate was oxygenated with 95% O2–5% CO2 and the partial pressure of O2 was kept at more than 600 mmHg.

Left ventricular pressure (LVP) was measured via a saline-filled latex balloon inserted into the left ventricle (LV) with a pressure transducer (TP-400T; Nihon Kohden, Tokyo, Japan). By adjusting balloon volume, the ventricle was loaded with 5–10 mmHg of initial LV end-diastolic pressure (LVEDP) and this balloon volume was maintained throughout the experiment. The first derivative of LVP (LVdp/dt) was derived from differentiating the signal of LVP electronically (ED-601G; Nihon Kohden). The LV developed pressure (LVPD) was obtained by subtracting LVEDP from LV systolic pressure. Mean coronary flow (CF) was measured with a flow probe (FF-030T; Nihon Kohden) attached to the aortic cannula, which was connected to an electromagnetic flow meter (MFV-3200; Nihon Kohden). After at least 20 min equilibration, the atrium was paced until the end of the experiment in order to eliminate the effects of changes in heart rate (HR). The pacing rate was set at 110% of its own HR (the mean±SEM HR was 339±9 b.p.m.; n=30).

Experimental protocol

The design of this experimental protocol is shown in Fig. 1. Hearts were randomly divided into non-ischaemic or ischaemic groups. Rats in the former group were infused with KHS (non-ischaemic KHS group; n=6), whereas those in the latter group were randomly divided into a further four groups that were infused with vehicle (KHS, Krebs–Henseleit solution; n=6) or landiolol at three concentrations of 30, 100 or 300 μmol/L (n=6 in each group). The concentrations of landiolol were set to be rather short. The reason for this is related to MDA measurements. Malondialdehyde can be washed out into myocardial interstitial effluent and the MDA tissue concentration decreases relative to prolongation of the period of reperfusion. Hence, the reperfusion time was set to be rather short.

Measurement of lactate dehydrogenase activity

Sampling of coronary effluent was performed at 20 min after the onset of reperfusion. These samples were stored at 4°C for measurement of lactate dehydrogenase (LDH) activity. Lactate dehydrogenase was estimated at 560 nm using an LDH monitoring kit (Lactate Dehydrogenase CI H Test Wako; Wako Pure Chemical, Osaka, Japan) and a spectrophotometer (UV-2200A; Shimadzu, Kyoto, Japan).

Measurement of myocardial tissue lipid peroxidation

At the end of the experiments, the LV free wall was quickly excised and frozen in liquid nitrogen. The extent of lipid peroxidation in the frozen myocardial tissues was measured by the thiobarbituric acid (TBA) method, with some modifications. The amount of TBA-reactive substances was estimated as MDA equivalents/g myocardial wet weight. The colour developed was measured at 532 nm. 1,1,3,3-Tetraethoxypypropane (TEP) was used as a standard.

Measurement of myocardial high-energy metabolites

Frozen myocardial sections were used for the determination of high-energy metabolites in myocardial tissues. After lyophilization for 6 h, dried tissues were homogenized with 0.6 mol/L perchloric acid. The mixture was centrifuged at 16 000 g for 15 min at 2°C and the supernatant was used for assay. Adenosine triphosphate was determined by the firefly luminescence method using an ATP monitoring agent (LL-100-2; Toyo Ink, Tokyo, Japan) and a lumiphotometer (Minilumat LB9506; Berthold, Calmbach, Germany). Creatine phosphate (CrP) and inorganic phosphate (Pi) were determined according to the method of Fiske and Subbarow as modified by Furchgott and De Gulbareff using a spectrophotometer (UV-150-02; Shimadzu).

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Experiments on isolated aortic ring preparations

In order to observe the direct action of landiolol on vascular smooth muscle, vascular preparations were obtained from the residual aorta of the rat used in the Langendorff experiments described above. As reported previously, the aorta was cut into a ring preparation of 2–3 mm width and suspended in an organ bath filled with KHS bubbled with 95% O2–5% CO2 at 37°C. Tension development of the aortic ring preparation was measured isometrically by means of a force-development transducer (TB-611T; Nihon Kohden). The preparation was precontracted with 30 mmol/L KCl and landiolol was added cumulatively.

Drugs

Landiolol hydrochloride was purchased from Ono Pharmaceutical (Tokyo, Japan); TBA and TEP were obtained from Sigma Chemicals (St Louis, MO, USA). All other chemicals were of analytical reagent grade. Landiolol was dissolved in KHS just prior to use.

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) followed by Fisher’s protected least significant difference post hoc test and paired observations were analysed with the paired t-test. All values are presented as the mean±SEM. The level for statistical significance was P < 0.05 or less.

RESULTS

In the non-ischaemic KHS group, none of the cardiovascular parameters showed any significant changes throughout the experiments (data not shown). Baseline values of cardiovascular parameters, such as LVEDP, LVDP, LVDp/dtmax, and CF, were similar in all ischaemic groups (Table 1). The effects of landiolol on the time-course of changes in the cardiovascular parameters are shown in Fig. 2. In the ischaemic KHS group, LVEDP increased during ischaemia and did not decrease to baseline levels during reperfusion. Values of LVDP, LVDp/dtmax and CF decreased immediately to almost zero and remained there throughout the period of ischaemia. The former two parameters showed partial recovery during reperfusion, whereas CF recovered completely. In the 30 μmol/L landiolol-treated group, LVEDP observed during reperfusion was significantly higher than that in the ischaemic KHS group and LVDP and LVDp/dtmax showed worse recovery. In addition, CF at 20 min after reperfusion was decreased to 77±4% of the baseline value and was significantly lower than that in the ischaemic KHS group. In the 100 μmol/L landiolol-treated group, all cardiovascular parameters measured were similar to those in the ischaemic KHS group. In the 300 μmol/L landiolol-treated group, LVDP and LVDp/dtmax observed during reperfusion were significantly higher than those in the ischaemic KHS group. Meanwhile, LVDP and LVDp/dtmax observed for the pre-ischaemic period were a little higher than those in the ischaemic KHS group, but the difference was not statistically significant.

The effects of landiolol on tissue concentrations of MDA at the end of reperfusion are shown in Fig. 3. The MDA in the 30 μmol/L landiolol-treated group was significantly higher than that in the non-ischaemic and ischaemic KHS groups. Conversely, the tissue content of MDA in the 300 μmol/L landiolol-treated group was the same as that in the non-ischaemic KHS group. Lactate dehydrogenase activity was minimal in the non-ischaemic group and that in the ischaemic groups was significantly increased. In the 30 μmol/L landiolol-treated group, LDH activity was significantly higher than that in the ischaemic KHS group (Fig. 4). The values of ATP were significantly low in all ischaemic groups, except for the 300 μmol/L landiolol-treated group, compared with the non-ischaemic KHS group. The ATP content in the 300 μmol/L

<table>
<thead>
<tr>
<th>Group</th>
<th>LVEDP (mmHg)</th>
<th>LVDP (mmHg)</th>
<th>LVDp/dtmax (mmHg/s)</th>
<th>CF (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischaemic KHS</td>
<td>9.9 ± 0.2</td>
<td>95 ± 5</td>
<td>3400 ± 190</td>
<td>9.4 ± 0.8</td>
</tr>
<tr>
<td>Landiolol</td>
<td></td>
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<tr>
<td>30 μmol/L</td>
<td>9.2 ± 0.5</td>
<td>92 ± 9</td>
<td>3100 ± 400</td>
<td>9.6 ± 0.6</td>
</tr>
<tr>
<td>100 μmol/L</td>
<td>10.5 ± 1.0</td>
<td>87 ± 3</td>
<td>3300 ± 310</td>
<td>9.7 ± 0.4</td>
</tr>
<tr>
<td>300 μmol/L</td>
<td>9.4 ± 0.5</td>
<td>83 ± 7</td>
<td>2900 ± 320</td>
<td>8.6 ± 1.0</td>
</tr>
</tbody>
</table>

Data are the mean±SEM (n = 6 in each group). There were no significant differences among values in each group.

LVEDP, left ventricular end-diastolic pressure; LVDP, left ventricular developed pressure; LVDp/dtmax, peak positive velocity of change of left ventricular pressure; CF, mean coronary flow; KHS, Krebs’–Henseleit solution.

Fig. 2 Effect of landiolol on the time-course of changes in (a) left ventricular (LV) end-diastolic pressure (LVEDP), (b) LV developed pressure (LVDP), (c) LVDp/dtmax and (d) coronary flow (CF). 100% corresponds to baseline values in Table 1. Time ‘0’ indicates the onset of ischaemia. Data are the mean±SEM (n = 6 in each group). *P < 0.05, **P < 0.01 compared with the ischaemic KHS group (●), KHS, Krebs’–Henseleit solution. (○), 30 μmol/L landiolol; (▲), 100 μmol/L landiolol; (□), 300 μmol/L landiolol.
landiolol-treated group was significantly higher than that in the ischaemic KHS group and was approximately the same as that in the non-ischaemic group (Fig. 4). In the 30 μmol/L landiolol-treated group, tissue concentrations of CrP were significantly lower than in the non-ischaemic KHS group. However, there was no significant difference in CrP content among the other ischaemic groups (Fig. 4). Conversely, the Pi content was significantly higher in all ischaemic groups, except for the 300 μmol/L landiolol-treated group, compared with the non-ischaemic KHS group. Compared with the ischaemic KHS group, the 300 μmol/L landiolol-treated group showed a significant decrement in Pi content (Fig. 4).

The effects of landiolol on isolated aortic preparations were observed during contracture with KCl 30 mmol/L. Landiolol, at 30 and 100 μmol/L, produced weak but statistically significant relaxations (94.9 ± 1.3 and 92.2 ± 1.4%, respectively), whereas 300 and 1000 μmol/L landiolol markedly relaxed preparations to 82.4 ± 1.3 and 42.4 ± 0.7% of control, respectively (Fig. 5). The vasorelaxant effect of 300 μmol/L landiolol was significantly greater \( (P < 0.001) \) than that of 30 μmol/L landiolol (Fig. 5).

**DISCUSSION**

The present study showed that landiolol has two opposing effects on ischaemia–reperfusion injuries in rat hearts: (i) a beneficial effect at a high concentration (300 μmol/L); and (ii) an exacerbating effect at a low concentration (30 μmol/L). Previously, Yasuda et al.\(^{15}\) reported that landiolol, up to 2.5 mmol/L, enhanced postischaemic cardiac function after warm cardioplegic arrest in rats. Their results may support those of the present study that a high concentration of landiolol can improve cardiac dysfunction after ischaemia. However, we should not equate the present results with those of Yasuda et al.,\(^{15}\) because their experimental protocol was different from ours: working mode was included in their protocol. Moreover, the concentrations of landiolol used in their study were over 30-fold higher than those used in the present study. Thus, the results of the two studies should be compared with great care.

![Fig. 3](image1.png)  
**Fig. 3** Effect of landiolol on malondialdehyde (MDA) content in the myocardium. Data are mean ± SEM (\( n = 6 \) in each group). ††† \( P < 0.001 \) compared with the non-ischaemic Krebs–Henseleit solution (KHS) group (NI); ** \( P < 0.01 \) compared with the ischaemic KHS group (I-KHS).

![Fig. 4](image2.png)  
**Fig. 4** Effect of landiolol on lactate dehydrogenase (LDH) activity in the coronary effluent 20 min after the start of reperfusion (a) and myocardium contents of (b) ATP, (c) creatine phosphate (CrP) and (d) inorganic phosphate (Pi) at the end of reperfusion. Data are the mean ± SEM (\( n = 6 \) in each group). † \( P < 0.05 \), †† \( P < 0.01 \), ††† \( P < 0.001 \) compared with the non-ischaemic Krebs–Henseleit solution (KHS) group (NI); * \( P < 0.05 \), ** \( P < 0.01 \), *** \( P < 0.001 \) compared with values obtained in the presence of 30 μmol/L landiolol.

![Fig. 5](image3.png)  
**Fig. 5** Effects of landiolol on isolated aortic ring preparations. Data are the mean ± SEM (\( n = 6 \) at each concentration) of percentage change. 100% corresponds to tension development obtained by 30 mmol/L KCl. ** \( P < 0.01 \), *** \( P < 0.001 \) compared with 100%; ††† \( P < 0.001 \) compared with values obtained in the presence of 30 μmol/L landiolol.
Conversely, Kurosawa et al. investigated the effects of landiolol (20–500 μmol/L) on ischaemia–reperfusion injuries using guinea-pig isolated and perfused hearts and reported cardioprotective effects of landiolol at concentrations of 100 and 500 μmol/L. Although similar concentrations of landiolol were used in the present study, the drug showed an aggravating effect at the low concentration (30 μmol/L) and a cardioprotective effect at the high concentration (300 μmol/L). The reasons for the differences between the two studies are suggested to be as follows: (i) differences in the species used; and (ii) differences in the experimental protocol. The most influential factor would be whether the isolated hearts were paced or not. The parameters measured in the study of Kurosawa et al. were HR, LVP and rate–pressure product and all experiments using guinea-pig isolated hearts were performed without pacing. Conversely, in the present study, rat isolated hearts were paced at a rate of 110% of their own heart beat and LVDP, LVdP/dt\text{max} and CF were measured. Thus, the hearts were paced in order to eliminate the effect of HR so that the effects of landiolol other than decreasing HR could be investigated.

In the present study, in addition to cardiodynamics, changes in high-energy metabolites and lipid peroxidation were determined. From these measurements, it was suggested that cardiac dysfunction obtained in the ischaemic KHS group may be due to a decrement of ATP content. Compared with the non-ischaemic group, the MDA content in the ischaemic KHS group was increased a little and so it was considered that lipid peroxidation did not affect cardiac dysfunction greatly. However, in the 30 μmol/L landiolol-treated group, ischaemia–reperfusion injuries were significantly aggravated; in addition to a decrement of myocardial ATP level, the tissue MDA content was increased significantly. These results mean that landiolol is able to enhance lipid peroxidation. This is supported by results from previous studies: acebutolol23 and pindolol24 enhanced the H2O2-induced lipid peroxidation. Thus, it was speculated that landiolol itself may be able to increase ROS production directly. In the 30 μmol/L landiolol-treated group, enhancement of lipid peroxidation through ROS production would damage the cellular membrane and increase intracellular Ca2+ influx, thus worsening the cardiac dysfunction induced by ischaemia.

Conversely, 30 μmol/L landiolol significantly relaxed isolated aortic preparations under KCl contracture. Because KCl contraction of vascular smooth muscle is mediated by activation of voltage-dependent Ca2+ channels,25 it was considered that landiolol has Ca2+ antagonist activity at a concentration of 30 μmol/L. This seemingly contradicts the present results that low concentrations of landiolol aggravate the ischaemia-induced cardiac dysfunction. However, the vasorelaxant effect with 30 μmol/L landiolol was significantly weaker (P < 0.001) than that with 300 μmol/L landiolol. Therefore, it was likely that the Ca2+ antagonist activity of 30 μmol/L landiolol was very weak and so could not inhibit the increase in intracellular Ca2+ influx followed by a decrement of ATP.

The results of the present study using isolated aortic preparations also indicated that landiolol, especially at concentrations of 300 μmol/L or more, has the possibility of competitive Ca2+ antagonistic activity. Similar results have been mentioned in a previous report; specifically, betaxolol, a selective β1-adrenoceptor antagonist, inhibited voltage-dependent Ca2+ channels in guinea-pig artery and vein.26 Thus, it is speculated that Ca2+ antagonistic activity was involved in ameliorating the effect of high concentrations of landiolol on cardiac dysfunction.

Measurement of myocardial high-energy metabolites revealed that the tissue content of ATP was significantly increased in the 300 μmol/L landiolol-treated group and was kept at the level seen in the non-ischaemic group. Because excess intracellular Ca2+ influx in the ischaemic myocardium provokes a decrement of ATP concentration, it is conceivable that high concentrations of landiolol can maintain ATP content through a Ca2+ antagonist action. In the 300 μmol/L landiolol-treated group, it was expected that ATP degradation was inhibited and thereby xanthine and hypoxanthine as substrates of xanthine oxidase were not produced. As a result, ROS production and lipid peroxidation may have been prevented.

The tissue content of MDA in the 30 μmol/L landiolol-treated group was the same as that in the non-ischaemic group. As stated above, landiolol itself would increase lipid peroxidation, but in the case of using high concentrations, it is expected that the Ca2+ antagonist action can outstrip the lipid peroxidation and improve ischaemia–reperfusion injuries.

In conclusion, the present study elucidated that landiolol has two different effects on ischaemia–reperfusion injuries in rat isolated hearts. Landiolol, at a low concentration (30 μmol/L), aggravates myocardial dysfunction with enhancement of lipid peroxidation through ROS production. Conversely, at a high concentration (300 μmol/L), landiolol ameliorates myocardial dysfunction by restoring ATP degradation and lipid peroxidation enhancement secondary to its Ca2+ antagonist action.

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