Propranolol induced lipid accumulation and mitochondrial granularity in myocardial cells

SVEIN ROTEVATN,* HARALD G JODALEN AND REIDAR K LIE

From the Cellular Cardiology Research Group, Institute of Anatomy, University of Bergen, Norway

SUMMARY The ultrastructure of the left ventricular wall was studied in mice given intraperitoneal injections of propranolol (5 mg or 20 mg·kg⁻¹ b.w. twice a day for 48 h with an additional dose 3 h before death), and in saline injected controls. Injections of propranolol increase the number of lipid droplets in areas of the myocardium. Fractional volume of lipid droplets in myocardial cells determined using quantitative stereological techniques, showed an increase of 280% and 544% in the group receiving the lowest and highest doses respectively. Injections of propranolol were also followed by an increased granularity of the mitochondria.

Propranolol is a widely used agent in a variety of clinical conditions. The clinical and pharmacophysiological properties of propranolol have been intensively studied during the last years. Yet, little attention has been paid to possible ultrastructural changes in myocardial cells brought about by this pharmacon.

In 1967 Sun et al¹ reported that injections of propranolol led to structural alteration in the mitochondria. They also noticed myelin bodies, overstretching of myofibrils and glycogen depletion. According to their report, the accumulation of lipid droplets occurred only rarely. However, Allin et al² found no consistent difference between sections from propranolol treated mice and control mice. Our preliminary ultrastructural studies indicated that propranolol treatment is followed by an increase in the number and size of lipid droplets in the myocardium. The aim of the present study is therefore to study the effect of propranolol injection on the fractional volume of myocardial lipid droplets, using quantitative stereology.

Materials and methods

Adult mice (NMR I strain) kept on a standard pellet diet with water ad libitum, were injected intraperitoneally with dl-propranolol HCl at two different dose levels, group I (six mice): 5 mg·kg⁻¹ b.w., group II (five mice): 20 mg·kg⁻¹ b.w. The two groups of mice were injected every 12th hour for 48 h with an additional dose 3 h before death. A group of

---

*Address for correspondence and reprint requests: Dr Svein Rotevatn, Institute of Anatomy, Årstadvei 19, University of Bergen, N-5000 Bergen, Norway.

Key words: Beta-blockade; cardiac muscle; lipid droplets; morphometric analysis.
control animals were injected with 0.9% sodium chloride solution at the same intervals.

The animals were killed by cervical dislocation, and the hearts were quickly excised and specimens from the left ventricular wall were fixed for 3.5 h in icecold Karnovsky’s fixative to which 5% sucrose was added. The specimens was thereafter washed overnight in a cacodylate-buffered 1% sucrose solution and then treated with 1% cacodylate-buffered OsO₄ solution for 1.5 h. After one wash in distilled water, the tissue was stained for 1.5 h in 2% uranyl acetate solution before dehydration through an acetone series. The ultrathin sections were grid-stained with lead citrate and thereafter examined in the electron microscope.

Five micrographs were randomly chosen from each of three different specimens from each animal and examined at a final magnification of \( \times 9500 \) for morphometric analysis. Volume fraction of lipid droplets of each micrograph was calculated according to the Delesse principle by a Kontron MOP AM 03 Morphometric Analyzer.

### Results

Micrographs from propranolol treated mice show scattered areas of increased number of lipid droplets when compared with saline injected controls (fig 1). Lipid droplets are spherical and located between myofibrils, often in close contact with mitochondria. This increment is not evenly distributed throughout the myocardium, but seems to be restricted to certain areas.

Estimates of fractional volume of lipid droplets in myocardial cells of propranolol treated and control mice are given in table 1. Group I shows an increase in the fractional volume of lipid droplets by 280%, whereas the corresponding increase in group II is 544%. Differences between the propranolol treated groups and the control group were tested for significance by analysis of variance (tables 2 and 3). It is evident from these tables that such significant differences exist.

In some areas the mitochondria of propranolol treated mice contain large numbers of electron dense

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD*</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>3.04 ( \cdot 10^{-3} )</td>
<td>2.09 ( \cdot 10^{-3} )</td>
<td>6</td>
</tr>
<tr>
<td>Control</td>
<td>0.80 ( \cdot 10^{-3} )</td>
<td>0.17 ( \cdot 10^{-3} )</td>
<td>6</td>
</tr>
<tr>
<td>Group II</td>
<td>5.09 ( \cdot 10^{-3} )</td>
<td>3.44 ( \cdot 10^{-3} )</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td>0.79 ( \cdot 10^{-3} )</td>
<td>0.19 ( \cdot 10^{-3} )</td>
<td>5</td>
</tr>
</tbody>
</table>

*Standard deviation of the mean of the animals

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>Mean square</th>
<th>F-ratio</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>1</td>
<td>226 ( 10^{-6} )</td>
<td>6.861</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Animals in groups</td>
<td>10</td>
<td>33.0 ( 10^{-6} )</td>
<td>6.434</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Specimens in animals</td>
<td>24</td>
<td>5.13 ( 10^{-6} )</td>
<td>1.527</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Fields in sections</td>
<td>144</td>
<td>3.36 ( 10^{-6} )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Level of significance.
TABLE 3  Analysis of variance of group II and control

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>Mean square</th>
<th>F-ratio</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>1</td>
<td>693 \cdot 10^{-6}</td>
<td>7.897</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Animals in groups</td>
<td>8</td>
<td>87.0 \cdot 10^{-6}</td>
<td>5.813</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Specimens in animals</td>
<td>20</td>
<td>15.1 \cdot 10^{-6}</td>
<td>4.018</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Fields in section</td>
<td>120</td>
<td>3.76 \cdot 10^{-6}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Level of significance.

Discussion

The present study shows that intraperitoneally injected propranolol brings about an increased fractional volume of lipid droplets in the heart and an increased mitochondrial granularity. These findings contrast with the report of Allin et al\(^2\) that no structural abnormalities could be related to the administration of the drug. In the latter study, the mice received the same dose levels as in group I, but only once a day, for 21 days, whereas in our study the drug was administered for only 48 h. The half-life of propranolol is known to be short,\(^6\) and the reason for the discrepancy might therefore be the comparatively long time passed from the last injection of propranolol until death in Allin’s study (1 day) as compared with the present study (3 h). Alternatively the ultrastructural changes might be transitory, having disappeared after 21 days.

Contrary to our findings, light microscopic observation by Sun et al,\(^1\) who used the same dose levels as Allin et al, indicated that accumulation of lipid droplets occurred only rarely. The former also found that injections of propranolol made the mitochondria appear swollen and vesicular with disrupted cristae, which is contrary to our results. Allin et al suggested that these features might be artificial as they tended to occur with equal frequency in their control group. The mitochondrial inclusions described by Sun et al seem different from the mitochondrial granules in our material as the latter appear smaller, more homogenous and more numerous.

An increased uptake of lipids in myocardial cells of propranolol treated mice would not be expected as propranolol has been shown to antagonise the isoprenaline induced increased activity of lipoprotein lipase in the heart\(^7\) and is known to inhibit catecholamine induced lipolysis in adipose tissue, thereby lowering plasma free fatty acids.\(^8\)\(^9\)

Regarding the effect of propranolol on myocardial metabolism, propranolol has been shown to enhance glycolysis\(^10\)\(^11\) and to influence lipid metabolism by reducing fatty acid oxidation and increasing the percentage conversion of [1-\(^14\)C] palmitat to tissue lipids.\(^10\)
Experiments carried out on isolated mitochondria from the heart indicate that when propranolol is added mitochondrial function is depressed. To what extent the depressed mitochondrial function contribute to changes in metabolism or to the increased mitochondrial granularity in this study is uncertain. The accumulation of lipid droplets in the myocardial cells of propranolol treated mice might therefore primarily be linked to a changed myocardial metabolism with reduced oxidation of fatty acids and increased glycolysis.

This work was supported by grants from The Norwegian Council on Cardiovascular Disease and from The Norwegian Research Council for Science and the Humanities.

References