Cardiovascular mortality is still among the most common causes of death. Several large clinical trials have confirmed that long-term β-blocker therapy reduced morbidity and mortality in patients with chronic heart failure as well as ischaemic disease. However, bradycardia and other cardiac disturbances after β-blockers therapy are the most dangerous side effects. Many patients with cardiovascular diseases receiving β-blockers are recommended to statin therapy, as well. Previous study showed that statins may desensitize β-adrenergic signaling, in cardiac myocytes via reduction of isoprenylation of G-protein γ-subunits. The aim of the study was to evaluate the influence of simvastatin at different doses and metoprolol injection on the heart rate in normocholesterolemic rats. The experiments were performed in Wistar rats, outbred males. Simvastatin at 1, 10 or 20 mg/kg or vehicle (0.2% methylcellulose) were given intragastrically during two-week period. After two week administration of simvastatin, rats were injected intraperitoneally with metoprolol at 5 mg/kg b.w. The heart rate signals were provided by Isotec pressure transducer connected to a direct current bridge amplifier and catheter was implanted into the right carotid artery. No changes in the baseline heart rate among all groups of the animals were observed. Metoprolol administration caused statistically significant decrease in heart rate in all groups of rats. In the control group, after metoprolol administration heart rate slowed down to 83.11 ± 1.11% (p < 0.05) of the baseline values, in group receiving simvastatin at 1 mg/kg b.w. 82.72 ± 5.49% (p < 0.05), in group receiving simvastatin at 10 mg/kg b.w. 85.13 ± 4.75 (p < 0.05) and in group receiving simvastatin at 20 mg/kg b.w. 85.13 ± 4.75% (p < 0.05) of the baseline values. No significant decrease in heart rate in the control group as compared to groups receiving simvastatin in different doses was observed. No significant changes among animals receiving simvastatin in different doses were observed, as well. Conclusion: Two week administration of simvastatin in different doses to normocholesterolemic rats does not modify metoprolol-induced depressing influence on the heart rate.

Keywords: rats, simvastatin, metoprolol, heart rate

INTERACTION BETWEEN DIFFERENT DOSES OF SIMVASTATIN AFTER TWO-WEEK ADMINISTRATION AND METOPROLOL INJECTION ON THE HEART RATE IN NORMOCHOLESTEROLEMIC RATS

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Abstract: Heart rate slowing is the beneficial effect after β-blocker administration in cardiac heart failure and ischemic heart disease. However, bradycardia and another cardiac disturbances after β-blockers therapy are the most dangerous side effects. Many patients with cardiovascular diseases receiving β-blockers are recommend- ed to statin therapy, as well. Previous study showed that statins may desensitize β-adrenergic signaling, in car- diac myocytes via reduction of isoprenylation of G-protein γ-subunits. The aim of the study was to evaluate the influence of simvastatin at different doses and metoprolol injection on the heart rate in normocholesterolemic rats. The experiments were performed in Wistar rats, outbred males. Simvastatin at 1, 10 or 20 mg/kg or vehi- cle (0.2% methylcellulose) were given intragastrically during two-week period. After two week administration of simvastatin, rats were injected intraperitoneally with metoprolol at 5 mg/kg b.w. The heart rate signals were provided by Isotec pressure transducer connected to a direct current bridge amplifier and catheter was implant- ed into the right carotid artery. No changes in the baseline heart rate among all groups of the animals were observed. Metoprolol administration caused statistically significant decrease in heart rate in all groups of rats. In the control group, after metoprolol administration heart rate slowed down to 83.11 ± 1.11% (p < 0.05) of the baseline values, in group receiving simvastatin at 1 mg/kg b.w. 82.72 ± 5.49% (p < 0.05), in group receiving simvastatin at 10 mg/kg b.w. 85.13 ± 4.75 (p < 0.05) and in group receiving simvastatin at 20 mg/kg b.w. 85.13 ± 4.75% (p < 0.05) of the baseline values. No significant decrease in heart rate in the control group as com- pared to groups receiving simvastatin in different doses was observed. No significant changes among animals receiving simvastatin in different doses were observed, as well. Conclusion: Two week administration of sim- vastatin in different doses to normocholesterolemic rats does not modify metoprolol-induced depressing influ- ence on the heart rate.

Keywords: rats, simvastatin, metoprolol, heart rate

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EXPERIMENTAL

The experiments were performed in forty five 8-11-week-old anesthetized Wistar rats, outbred males. The study was approved by the Ethics Committee of the Medical University of Lodz (Poland)- L/BD/300.

Animals

A several day adaptation period was scheduled prior to the beginning of the experiment. After adaptation, simvastatin (Polfarmex, Poland series no. KY-SI-M20030102) in doses of 1, 10 and 20 mg/kg b.w. or vehicle (0.2% methylcellulose) were given intragastrically (i.g.) over two week period. Rats had free access to standard diet (granulated MIX*LSK*) and water.

Surgical procedures and heart rate recording

The surgery was performed 24 h after the administration of the last drug dose and 10 h after the last feed administration. For further surgical procedures, anesthesia was initiated by an intraperitoneal (i.p.) dose of pentobarbital sodium at 60 mg/kg b.w. The anesthesia was maintained by intravenous bolus injections of pentobarbital sodium at 10 mg/kg b.w. as needed. For the heart rate measurement catheters were implanted into the right carotid artery. The heart rate signals were provided by an Isotec pressure transducer connected to a direct current bridge amplifier (both Hugo Sachs Elektronik).

Experimental protocol

The experimental protocol was performed in anesthetized rats and included single i.p. injection of metoprolol at 5 mg/kg b.w. or 0.9% NaCl (2 mL/100 g b.w.). Metoprolol or 0.9% NaCl administration was followed by hemodynamic stabilization period (about 20 min). After heart rate assessment 0.25 mL of blood samples were taken for further lipid profile examination.

Data analysis

For each rat baseline recordings were obtained. In each of these data files, the signals for heart rate were stored at a sampling rate of 500 Hz. Heart rates were calculated on a beat-by-beat basis.

Stable baseline conditions throughout the experimental protocol were confirmed by comparing the parameters during the individual baseline recordings. The results of the metoprolol injection are given as the absolute differences from baseline for heart rate and as percent changes from baseline.

Statistics

All data were presented as the means ± SD (standard deviation). Statistical comparisons between baseline conditions and metoprolol injection were done by paired Student’s t-test. Comparisons between the groups were performed using ANOVA. Post-hoc comparisons were performed using LSD test. Normal distribution of parameters was checked by means of Shapiro-Wilks test. If data were not normally distributed or the val-

<table>
<thead>
<tr>
<th></th>
<th>before metoprolol or 0.9% NaCl injection</th>
<th>after metoprolol or 0.9% NaCl injection</th>
<th>difference [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_Na</td>
<td>455.31 ± 16.08 (5)</td>
<td>455.25 ± 21.43</td>
<td>100.04 ± 4.75</td>
</tr>
<tr>
<td>K_M</td>
<td>425.11 ± 27.49 (5)</td>
<td>354.52 ± 27.70</td>
<td>83.65 ± 1.11†</td>
</tr>
<tr>
<td>S1_Na</td>
<td>434.98 ± 41.62 (8)</td>
<td>428.46 ± 46.28</td>
<td>98.44 ± 3.57</td>
</tr>
<tr>
<td>S1_M</td>
<td>429.27 ± 34.11 (5)</td>
<td>364.64 ± 34.72</td>
<td>84.89 ± 2.53†</td>
</tr>
<tr>
<td>S10_Na</td>
<td>458.43 ± 15.53 (5)</td>
<td>457.72 ± 11.25</td>
<td>99.87 ± 1.43†</td>
</tr>
<tr>
<td>S10_M</td>
<td>462.43 ± 31.65 (5)</td>
<td>381.53 ± 17.85</td>
<td>82.72 ± 5.42†</td>
</tr>
<tr>
<td>S20_Na</td>
<td>456.74 ± 27.34 (5)</td>
<td>457.58 ± 25.62</td>
<td>100.19 ± 0.41</td>
</tr>
<tr>
<td>S20_M</td>
<td>465.99 ± 24.99 (7)</td>
<td>396.58 ± 29.19</td>
<td>85.13 ± 4.75†</td>
</tr>
</tbody>
</table>

K_Na indicates control group receiving 0.9% NaCl injection, K_M indicates control group receiving metoprolol injection, S1_Na, S1_M – indicate rats receiving 1 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, S10_Na, S10_M – indicate rats receiving 10 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, S20_Na, S20_M – indicate rats receiving 20 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, (n) – number of rats in group, † indicates p < 0.05 t-par.
Interaction between different doses of simvastatin after two-week...

VALUES OF VARIANCE WERE DIFFERENT, ANOVA WITH KRUSCAL-WALLIS AND MANN-WHITNEY’S U TEST WERE USED. ALL PARAMETERS WERE CONSIDERED STATISTICALLY SIGNIFICANTLY DIFFERENT IF P < 0.05. THE STATISTICAL ANALYSIS OF HEART RATE AND HEMODYNAMIC PARAMETERS WAS PERFORMED USING STATGRAPHICS 5.0 PLUS SOFTWARE.

RESULTS

THE EXEMPLARY HEART RATE RECORDINGS BEFORE AND AFTER METOPROLOL ADMINISTRATION IS SHOWN IN FIGURE 1. NO CHANGES IN THE BASELINE HEART RATE, I.E. BEFORE METOPROLOL OR 0.9% NaCl INJECTION, AMONG ALL GROUPS OF ANIMALS WERE OBSERVED. THE ADMINISTRATION OF 0.9% NaCl DID NOT INFLUENCE RAT HEART RATE. METOPROLOL ADMINISTRATION CAUSED STATISTICALLY SIGNIFICANT DECREASE IN HEART RATE IN RATS RECEIVING VEHICLE, AS WELL AS SIMVASTATIN. IN THE CONTROL GROUP, AFTER SINGLE METOPROLOL ADMINISTRATION HEART RATE SLOWED DOWN TO 83.11 ± 1.11% OF THE BASELINE VALUES, IN GROUP RECEIVING SIMVASTATIN AT 1 mg/kg b.w. 82.72 ± 5.49%, simvastatin at 10 mg/kg b.w. 85.13 ± 4.75 and in group receiving simvastatin at 20 mg/kg b.w. heart rate slowed down to 85.13 ± 4.75% of the baseline values. NO SIGNIFICANT DECREASE IN HEART RATE IN THE CONTROL GROUP AS COMPARED TO GROUPS RECEIVING SIMVASTATIN IN DIFFERENT DOSES WAS OBSERVED. NO SIGNIFICANT CHANGES AMONG

Table 2. Total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides (the mean ± SD) in rats during two week administration of simvastatin or placebo [mmol/L].

<table>
<thead>
<tr>
<th></th>
<th>TCH</th>
<th>HDL-CH</th>
<th>LDL-CH</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_Na</td>
<td>1.23 ± 0.07</td>
<td>0.20 ± 0.03</td>
<td>1.02 ± 0.07</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>K_M</td>
<td>1.28 ± 0.07</td>
<td>0.19 ± 0.03</td>
<td>1.07 ± 0.08</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>S1_Na</td>
<td>1.38 ± 0.09</td>
<td>0.23 ± 0.04</td>
<td>1.13 ± 0.10</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>S1_M</td>
<td>1.43 ± 0.06</td>
<td>0.21 ± 0.03</td>
<td>1.21 ± 0.06</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>S10_Na</td>
<td>1.34 ± 0.05</td>
<td>0.21 ± 0.02</td>
<td>1.11 ± 0.04</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>S10_M</td>
<td>1.39 ± 0.07</td>
<td>0.23 ± 0.03</td>
<td>1.14 ± 0.07</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>S20_Na</td>
<td>1.22 ± 0.12</td>
<td>0.17 ± 0.03</td>
<td>1.04 ± 0.11</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>S20_M</td>
<td>1.22 ± 0.10</td>
<td>0.22 ± 0.03</td>
<td>0.99 ± 0.09</td>
<td>0.26 ± 0.03</td>
</tr>
</tbody>
</table>

K_Na indicates control group receiving 0.9% NaCl injection, K_M indicates control group receiving metoprolol injection, S1_Na, S1_M – indicate rats receiving 1 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, S10_Na, S10_M – indicate rats receiving 10 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, S20_Na, S20_M – indicate rats receiving 20 mg/kg simvastatin and 0.9% NaCl or metoprolol injection.

Figure 1. Heart rate after metoprolol administration in rat.
animals receiving simvastatin in different doses were observed, as well (Table 1).

Changes in plasma total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides levels (the mean ± SD) after two week administration of simvastatin or vehicle [mmol/L] are presented in Table 2.

**DISCUSSION AND CONCLUSION**

Selective β-blockers are drugs with multiple cardiac actions. The blockade of β-1 receptors results in slowing of heart rate, reduction in myocardial contractility and lowering of systemic blood pressure. The use of early β-blocker therapy in acute myocardial infarction reduces the risks of reinfarction and ventricular fibrillation, however, it increases the risk of cardiogenic shock (18). Only several reports indicate the possible interaction between statins and β-blockers. For example, it has been demonstrated that concomitant administration of fluvastatin and propranolol could protect from dangerous incidents of cardiac arrhythmia in rats (19). On the other hand also statins given in monotherapy could possess benefit antiarrhythmic effects. The AVID study revealed that statins significantly decreased the occurrence of the ventricular tachycardia and ventricular fibrillation (20). The mentioned above positive impact of co-administered fluvastatin and propranolol is suggested to result from statin influence on the autonomic nervous system. The above hypothesis was confirmed by Pliquett et al. They showed that simvastatin in rats restored the sympathetic–parasympathetic balance (15). Additionally, Philip et al. suggested that the impact of statins on the autonomic nervous system is most probably the effect of extralipid action of simvastatin (16). Statins reduce the isoprenoid cholesterol intermediates and as well as dolichols, geranylgeranoic acid and farnesyl-farnesoic acid (21) and it was shown that statin influences the β-adrenergic stimulation which is connected with their impact on isoprenylation of G-protein γ-subunits. By interfering with Gγ isoprenylation, statins could influence membrane association and function of heterotrimeric G-proteins (22, 23). This effect was accompanied by cytosolic accumulation of a fraction of G-protein γ-subunits and a decrease in Gα total protein. The statin effect on Gαγ was fully reversed by mevalonate and GGPP, but not by FPP or squalene, which seems to confirm the contribution of statin impact on isoprenylation process rather than direct lipid-lowering activity to development of the described changes (17). Biochemical evidence for such an effect has been reported earlier (22, 24). In our study, short term administration of simvastatin to normocholesterolemic rats did not modify metoprolol-induced depressing influence on the heart rate. This observation in normocholesterolemic rats was independent of the dose of drug used in the experiment. Also previous studies performed in humans (25, 26) did not reveal apparent “anti-adrenergic effects” of statins such as a reduction of heart rate. Probably it is connected with duration of simvastatin administration and/or hyperlipidemir disturbances. The next studies in another models are needed for further assessment of interaction between statins and β-blockers.

**Acknowledgments**

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**REFERENCES**