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Dobutamine as selective β₁-adrenoceptor agonist in in vivo studies on human thermogenesis and lipid utilization


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Schiffelers, S. L. H., V. J. A. van Harmelen, H. A. J. de Grauw, W. H. M. Saris, and M. A. van Baak. Dobutamine as selective β₁-adrenoceptor agonist in in vivo studies on human thermogenesis and lipid utilization. J. Appl. Physiol. 87(3): 977–981, 1999.—The use of dobutamine as selective β₁-adrenoceptor agonist in vivo studies on human thermogenesis and lipid utilization was investigated in 20 men. At 2.5, 5, and 10 µg·kg⁻¹·min⁻¹, dobutamine induced significant increases in energy expenditure, lipid oxidation, and lipolysis. The β₁-adrenoceptor antagonist atenolol (bolus: 42.5 µg/kg, infusion: 1.02 µg·kg⁻¹·min⁻¹) blocked all dobutamine-induced effects on thermogenesis and lipid utilization. All parameters remained at levels comparable to those during saline infusion. The dose of atenolol used did not inhibit β₂-adrenoceptor-specific changes in energy expenditure, lipid oxidation, and lipolysis during salbutamol infusion (85 ng·kg⁻¹·min⁻¹). This indicates that atenolol was specific for β₁-adrenoceptors and did not camouflage concomitant β₂-adrenoceptor stimulation during dobutamine infusion. Therefore, we conclude that dobutamine can be used as a selective β₁-adrenoceptor agonist at doses ≤10 µg·kg⁻¹·min⁻¹ in in vivo studies on human thermogenesis and lipid utilization.

Atenolol; salbutamol; lipid oxidation; lipolysis

The sympathetic nervous system plays an important role in the regulation of human thermogenesis. Sympathetic nervous system activity is mainly stimulated in response to food digestion and physical exercise but can also be triggered by cold exposure or pathogenic stimuli. In response to these stimuli, catecholamines are released that subsequently induce thermogenesis (14). This increase in energy expenditure is due to stimulation of both β₁- and β₂-adrenoceptors of the sympathetic nervous system (4). α₁-Adrenoceptors probably do not play a role (4, 6, 17). The effect of β₂-adrenergic stimulation on human thermogenesis is, at the moment, still debatable (4, 11, 23), because the available agonists appear to be only weak partial agonists in humans (1). In rodents β₁-agonists induce significant effects, but this might be explained by the pharmacological differences between human and rodent β₁-adrenoceptors (10, 15).

In obese men nonselective β-adrenergic stimulation leads to a reduced increase in thermogenesis and lipid utilization compared with in lean men (3). Therefore, it is interesting to know whether these impaired responses might be due to a defect in the β₁- or the β₂-adrenoceptor. The most selective β₁-adrenoceptor agonist for in vivo use in humans is dobutamine. In lean healthy volunteers dobutamine increases oxygen consumption, indicating an increase in thermogenesis (2, 7), decreases respiratory exchange ratio (RER), suggesting an increase in lipid oxidation, and increases plasma glycerol and nonesterified fatty acids (NEFA) concentrations, indicating an increase in lipolysis (7).

However, both in vitro (15, 16) and in vivo (12, 13) animal studies have shown that dobutamine also has α₁- and β₂-adrenoceptor agonistic properties. Because α₁-adrenoceptors are not important for human thermogenesis, their role was not further investigated. The selectivity of dobutamine for β₁- and β₂-adrenoceptors in studies on human thermogenesis and lipid utilization was elucidated in this study. Therefore, we evaluated the effect of atenolol, a predominantly β₁-adrenoceptor antagonist, on dobutamine-induced increases in energy expenditure, lipid oxidation, and lipolysis. Addition of atenolol should block all β₁-adrenoceptor-mediated effects and reveal all other effects of dobutamine. In a control test, the selective β₂-adrenoceptor-blocking properties of atenolol at the dose used were verified. Addition of atenolol should have no effect on the increases in thermogenesis and lipid utilization induced by the selective β₂-adrenoceptor agonist salbutamol.

MATERIALS AND METHODS

Subjects. Twenty lean male volunteers participated in this study. Mean age and body mass index were 22.0 yr (range: 18–27 yr) and 21.9 kg/m² (range: 19.4–25.3 kg/m²), respectively. The subjects were healthy and took no medication at the time of the study. They gave written informed consent before participating in the study. The study protocol was reviewed and approved by the Ethics Committee of Maastricht University.

Experimental protocol. The study protocol consisted of four tests. In the dobutamine test, a 30-min baseline period was followed by consecutive infusions of 2.5, 5, and 10 µg·kg⁻¹·min⁻¹ dobutamine (selective β₁-adrenoceptor agonist; Dobax, Byk, Zwangenburg, The Netherlands), each dose administered during 30 min. This test intended to measure all dobutamine-mediated effects. The saline test consisted of a 30-min baseline period followed by three times a 30-min period of saline infusion (0.6 ml/min) to study the effects of saline compared with the baseline period. The test infusion consisted of a 30-min baseline period followed by three times a 30-min period of saline infusion (0.6 ml/min) to study the effects of saline compared with the baseline period. The dose used was verified. Addition of atenolol should have no effect on the increases in thermogenesis and lipid utilization induced by the selective β₂-adrenoceptor agonist salbutamol.

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which a continuous infusion of atenolol (1.02 µg·kg\(^{-1}\)·min\(^{-1}\)) was started for the remainder of the test. The salbutamol plus atenolol test consisted of a 45-min baseline period, after which the β\(_2\)-adrenoceptor agonist salbutamol (Ventolin, GlaxoWellcome, Zeist, The Netherlands) was given for 90 min at an infusion rate of 0.85 ng·kg\(^{-1}\)·min\(^{-1}\). During the last 45 min, atenolol was added to the salbutamol infusion at the same dose as described above to study possible β\(_2\)-adrenoceptor-blocking effects of atenolol. The infusion periods were prolonged during the last test, because thermogenesis did not reach steady state within 30 min during salbutamol infusion, as it did during dobutamine infusion. Twenty subjects participated in the dobutamine test, 10 subjects in the saline test, 14 subjects in the dobutamine plus atenolol test, and 10 subjects underwent the salbutamol plus atenolol test. Each of the 20 subjects participated in 2 or 3 trials. There were no statistically significant differences in subject parameters between tests. The study design was single blind, and the order of tests was randomized.

The subjects came to the laboratory at 8:30 AM, with at least 2 days between tests. All individuals were fasted for at least 10 h (overnight) and came to the laboratory by car or by bus to minimize the amount of physical activity before the tests. At the beginning of each test, a catheter was inserted into a forearm vein for drug infusion and blood sampling. During the tests, energy expenditure and RER were continuously measured and, at the end of each 30- or 45-min interval, a blood sample was obtained. For safety reasons the infusion was stopped when heart rate had increased >30 beats/min and/or mean blood pressure had risen more than 30 mmHg. After these criteria, one subject was not tested at the highest dose of 10 µg·kg\(^{-1}\)·min\(^{-1}\) dobutamine. Room temperature was kept between 23 and 25°C.

Methods. An open-circuit ventilated-hood system was used for measurement of whole body energy expenditure and RER. The volume of air drawn through the hood was measured by a dry-gas meter (Schlumberger, Dordrecht, The Netherlands), and the composition of the inflowing and outflowing air was analyzed by a paramagnetic O\(_2\) analyzer (Servomex, Crowborough, UK) and an infrared CO\(_2\) analyzer (Hartmann and Braun, Frankfurt, Germany). Airflow rate and the O\(_2\) and CO\(_2\) concentrations of the inflowing and outflowing air were used to compute O\(_2\) consumption (coefficient of variation 2.4%) and CO\(_2\) production (coefficient of variation 3.1%) on-line every 2 min through an automatic acquisition system interfaced with a personal computer. Energy expenditure was calculated according to the formula of Weir (21). Energy expenditure and RER values were averaged over the last 10 min of each infusion step, during which their values were stable, and their means were used in the data analysis. Blood pressure was measured by an automated blood pressure device (Tonoprint, Speidel & Keller, Jungingen, Germany, and UA 731, Takeda Medical, Rotterdam, the Netherlands) during the last 10 min of each period. The mean of four measurements per interval was computed and used for further analysis. Heart rate was monitored continuously by conventional electrocardiogram and was recorded at the end of every 5-min period. The values over the last 10 min were averaged and used for further analysis.

Analytic methods. Blood samples for glycerol and NEFA determination were preserved in sodium-EDTA. All samples were immediately centrifuged for 1 min at 7,280 g. Plasma was transferred to microtest tubes, rapidly frozen in liquid nitrogen, and stored at −70°C until further analysis. Plasma glycerol concentrations were measured with a glycerol kit (Boehringer 148270, Mannheim, Germany), and plasma NEFA concentrations were measured with the NEFA C kit (Wako NEFA C kit 99475409, Neuss, Germany), both on a Cobas-Fara analyzer (Roche Diagnostica, Basel, Switzerland). In each run, standard samples with known concentrations were included for quality control.

Data analysis. All values are presented as means ± SE. The differences in outcome between the dobutamine, the saline, and the dobutamine plus atenolol tests were analyzed with a split-block incomplete-block factorial ANOVA. In this design, categories are made for treatment and subject to account for missing values. Post hoc testing between studies was done according to Bonferroni’s inequalities. The effects within studies were analyzed with repeated-measurements ANOVA. Post hoc testing between time points was done with a paired t-test, corrected according to Bonferroni’s inequalities. All statistical tests were performed two sided. P < 0.05 was regarded as statistically significant.

RESULTS

Dobutamine, saline, and dobutamine plus atenolol tests. Energy expenditure increased significantly during dobutamine infusion (P < 0.001) (Fig. 1). During
saline infusion there was no significant change in energy expenditure. Simultaneous administration of atenolol completely prevented the dobutamine-induced increase in energy expenditure. Energy expenditure remained at a similar level as during the saline test. RER decreased significantly in all three tests (dobutamine, saline: \( P < 0.001 \); dobutamine plus atenolol: \( P < 0.01 \)) (Fig. 1). During the second and third infusion period of dobutamine, RER was significantly lower than during the corresponding infusion periods with saline (both \( P < 0.001 \)). Atenolol infusion prevented the more pronounced reduction in RER at the higher dobutamine dosages. RER decreased to a comparable level as during saline infusion.

Plasma glycerol and NEFA concentrations increased significantly with dobutamine (both \( P < 0.001 \)), saline (glycerol: \( P < 0.01 \), NEFA: \( P < 0.001 \)), as well as
Table 1. Parameters at baseline, during salbutamol infusion, and during salbutamol plus atenolol infusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Salbutamol (85 ng·kg⁻¹·min⁻¹)</th>
<th>Salbutamol Plus Atenolol (1.02 µg·kg⁻¹·min⁻¹)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy expenditure, kJ/min</td>
<td>5.40 ± 0.19</td>
<td>6.20 ± 0.14p</td>
<td>5.95 ± 0.20p</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>0.83 ± 0.01</td>
<td>0.82 ± 0.01</td>
<td>0.83 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Glycerol concentration, µmol/l</td>
<td>53.6 ± 4.7</td>
<td>115.2 ± 14.2a</td>
<td>96.7 ± 13.1c,g</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NEFA concentration, µmol/l</td>
<td>249 ± 32</td>
<td>710 ± 75p</td>
<td>472 ± 65g</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>56 ± 2</td>
<td>70 ± 3p</td>
<td>65 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>117 ± 3</td>
<td>130 ± 3p</td>
<td>121 ± 4p</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>67 ± 2</td>
<td>61 ± 2</td>
<td>65 ± 3</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 subjects. NEFA, nonesterified fatty acids; NS, not significant. Atenolol was bolus administered at 42.5 µg/kg.

Paired t-test: rest vs. salbutamol: aP < 0.01, bP < 0.001; salbutamol vs. salbutamol plus atenolol: cP < 0.05, dP < 0.01, eP < 0.001; rest vs. salbutamol plus atenolol: fP < 0.05, gP < 0.01, hP < 0.001.
salbutamol for β2- and β1-adrenoceptors lies only eightfold apart (8). This suggests that concomitant β1-adrenoceptor stimulation during salbutamol infusion is more likely to have occurred than β2-adrenoceptor blockade during simultaneous atenolol infusion. The significant decreases in plasma glycerol and NEFA concentrations after the addition of atenolol might therefore be due to the blockade of the β2-adrenoceptor-mediated effects of salbutamol. Another explanation might be that atenolol blocked the basal β2-adrenoceptor-mediated effects of the endogenous catecholamines on lipolysis.

It is still uncertain which processes are responsible for sympathetically mediated thermogenesis and in which tissues these processes are localized. Several authors (9, 18) have suggested that the catecholamine-induced increase in whole body energy expenditure may partly be explained by the increase in myocardial energy expenditure caused by an increase in cardiac output. Myocardial energy expenditure can be estimated by the rate-pressure product (heart rate × systolic blood pressure) (20). In our study, the estimated increase in myocardial energy expenditure would result in an overall increase in energy expenditure of 14% during the dobutamine test and of 2% during the dobutamine plus atenolol test. Whole body energy expenditure, however, increased 33% during the dobutamine test and 5% during the dobutamine plus atenolol infusion. The majority of the increase in energy expenditure, therefore, appeared to result from substrate oxidation in other tissues.

In summary, the results of this study indicate that, at dosages of 2.5, 5, and 10 µg·kg⁻¹·min⁻¹, the predominantly β1-adrenoceptor agonist dobutamine caused significant increases in energy expenditure, lipid oxidation, and lipolysis. The β1-adrenoceptor-antagonist atenolol blocked all dobutamine-induced increases in thermogenesis and lipid utilization. All parameters remained at levels comparable with those during saline infusion. The dose of atenolol used was specific for β1-adrenergic blockade and therefore did not camouflage concomitant β2-adrenoceptor stimulation by dobutamine. Therefore, we conclude that dobutamine can be used as selective β1-adrenoceptor agonist at dosages ≤10 µg·kg⁻¹·min⁻¹ in vivo studies on human thermogenesis and lipid utilization.

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