The effect of a neuropeptide Y Y1 receptor antagonist in patients with angina pectoris

L. Gullestad, T. Bjurø, L. Aaberge, T. Apelland, R. Skårdal, E. Kjekshus, M. Nordlander, B. Åblad, J. Pernow

Department of Cardiology Rikshospitalet University Hospital, Oslo, Norway
Wallenberg Laboratory, Sahlgrens University Hospital, Gothenburg, Sweden
AstraZeneca R & D, Möln达尔, Sweden
Department of Cardiology, Karolinska Hospital, Stockholm, Sweden

Received 27 November 2002; revised 21 February 2003; accepted 20 March 2003

Aims Neuropeptide Y (NPY) is a potent vasoconstrictor released during sympathetic activation that may be involved in myocardial ischaemia. We examined the effect of a Y1 receptor antagonist on haemodynamic and ischaemic responses to exercise in patients with coronary artery disease.

Methods and results Eighty-two evaluable male patients were included in a randomized, double blind, two-way crossover study with a low dose (6.7 µg/kg/min; n=59) and a high dose (13.3 µg/kg/min; n=23) of the Y1 receptor antagonist AR-H040922 given as infusions for 2 h or placebo. Myocardial ischaemia during a symptom-limited exercise test was monitored by conventional ST-segment analysis and heart rate (HR)-adjusted ST changes including the ST/HR slope and ST/HR recovery. Administration of the high dose AR-H040922 attenuated systolic blood pressure by 6–11 mmHg (p<0.05) during and after exercise without affecting HR. None of the two doses of AR-H040922 influenced any of the ischaemic parameters or duration of exercise, however. The maximal increase in NPY was higher during AR-H040922 (p<0.05) compared with placebo.

Conclusions Selective NPY Y1 receptor blockade attenuates the increase in blood pressure during exercise indicating a role for endogenous NPY in blood pressure regulation. Despite this effect, the Y1 receptor antagonist did not influence exercise-induced ischaemic parameters in patients with coronary artery disease.

© 2003 The European Society of Cardiology. Published by Elsevier Science Ltd. All rights reserved.

KEYWORDS
Coronary artery disease; exercise testing; myocardial ischaemia; peptides

Introduction
Myocardial ischaemia during physical exercise in patients with angina pectoris is caused by oxygen demand exceeding the supply, and is mainly due to the activation of the sympathetic nervous system. Release of noradrenaline leads to increased myocardi...
exercise in patients with angina pectoris and could contribute to myocardial ischaemia, especially in the recovery phase after exercise.³

NPY is particularly abundant in the perivascular sympathetic nerve fibres, where it is co-stored and released with noradrenaline.⁴ Its cardiovascular effects are multiple and not fully explored, but a long-lasting vasoconstriction, which is not mediated via /α/-adrenergic receptors, is prominent.⁴ In anaesthetized dogs, myocardial release of NPY elicited by cardiac sympathetic nerve stimulation correlated significantly with the coronary vasoconstrictor response which persisted after /α/- and /β/-adrenoceptor blockade.⁵ Intracoronary infusion of NPY in patients with angina pectoris induces myocardial ischaemia with typical chest pain and ECG-changes.⁶ This may indicate that NPY is a mediator of coronary vasoconstriction also in man.

NPY mediates its effects through several receptor subtypes. It is believed that NPY exerts direct vasoconstrictor effects via activation of the Y1 receptor.⁷ In addition to the direct effects, NPY has been demonstrated to potentiate the vasoconstrictor response to noradrenaline.⁸ Accordingly, NPY (Y1) receptor antagonists attenuate both the direct and indirect vasoconstrictor responses evoked by NPY.⁷

The hypothesis of the present study was that NPY contributes to myocardial ischaemia during exercise in patients with angina. Therefore, we examined the influence of the selective Y1 antagonist AR-H040922⁹ on haemodynamic changes and signs of myocardial ischaemia both during and after exercise in patients with stable angina pectoris.

**Methods**

**Patients**

Eighty-eight male patients between 30–75 years of age, with chronic stable angina pectoris for at least 3 months, with positive (≥1 mm ST segment depression) symptom limited exercise test, who were referred for coronary angiography for suspected coronary artery disease, were recruited for the study. In six of these patients >1 mm ST segment depression was not obtained during the study exercise. These patients were therefore not included. The final study thus included 82 patients. Stable angina was defined as angina that had not changed in characteristics during the last four weeks before inclusion. Significant coronary artery disease was confirmed in all patients, except nine who eventually refused angiography. All patients were in sinus rhythm, with stable clinical condition, without myocardial infarction during the last 3 months, and with unchanged medication (Table 1) during the last month. Excluded from participation were patients with clinically significant peripheral vascular or cerebral disease, insulin dependent-diabetes mellitus, renal insufficiency (creatinine >180 µmol/l), clinically significant liver disease, pulmonary disease or patients using digitalis. Informed consent was obtained from all participants and the Ethics Committee of the hospital (Rikshospitalet, University of Oslo, Norway) approved the protocol.

**Design**

The study was performed as a randomized, double blind, two-way crossover, and placebo-controlled study in two separate groups of patients. One group of patients (n=64) received a low dose of the NPY antagonist AR-H040922 at a dose rate of 6.7 µg/kg/min or and placebo (NaCl) on two occasions. The other group (n=24) received a high dose of AR-H040922 at a rate of 13.3 µg/kg/min and placebo. AR-H040922 and placebo were given as i.v. infusions for 120 min. The washout period between the two occasions was at least 1 week. Exercise started 45 min after start of the infusion.

**Exercise testing**

The subjects were instructed not to take part in physical training the day before the exercise test, and to eat a normal mixed diet. They were not allowed to eat, drink coffee or smoke during the last 4 h before the study, and the morning medication was postponed until after the exercise test. The two tests were performed at the same time of the day and under similar conditions.

The bicycle exercise test was carried out on an electrically braked ergometer cycle with a constant pedal rate of 60 rpm. Workload was initiated at

### Table 1 Clinical characteristics of the study population (n=82)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60±8</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>17</td>
</tr>
<tr>
<td>Cardiac failure (%)</td>
<td>2</td>
</tr>
<tr>
<td>Medication for angina (%)</td>
<td></td>
</tr>
<tr>
<td>β-blockers (%)</td>
<td>94</td>
</tr>
<tr>
<td>Ca-blocker (%)</td>
<td>–</td>
</tr>
<tr>
<td>Long acting nitrates (%)</td>
<td>47</td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>91</td>
</tr>
<tr>
<td>Lipid lowering drugs (%)</td>
<td>87</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>19</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>71±13</td>
</tr>
<tr>
<td>Left ventricular end diastolic pressure (mmHg)</td>
<td>15±5</td>
</tr>
</tbody>
</table>
40 W with subsequent increments of 10 W every minute until they stopped because of exhaustion, angina pain, dyspnoea or the occurrence of a systolic blood pressure drop of >10 mmHg. Heart rate and blood pressure were recorded before start of the infusion, 45 min after start of infusion, every second minute during exercise, at maximal exercise, and at 1, 2, 4, 10 and 30 min in the post-exercise period. Blood pressure was determined by the auscultatory method with a blood pressure cuff placed around the upper arm. Heart rate was measured from the ECG recording. After the test the patient remained seated for 1 min on the bicycle and thereafter laid down for the rest of the period.

A 12-lead ECG was continuously sampled into a computer before, during and until 10 min after exercise. Special care was taken to reduce noise in the analogue ECG by skin preparation and electrode handling. The ST-amplitude was measured 60 ms (ST60) after the end of the QRS complex and signal averaging was performed in consecutive 10 s intervals so that six ST-values were obtained each minute in 12-leads throughout the entire test. The main variable was time to >1 mm ST-depression in lead V5. The 1 mm level during exercise was calculated by the computer using linear regression over 2 min and related to an average of 12 values at rest before exercise. Secondary variables were absolute ST amplitude changes, the ST/HR slope and the ST-'deficit'. Absolute ST amplitude values were measured in all 12 leads as an average of data obtained during the last 30 s of exercise. A ST/HR slope was calculated by linear regression in all leads during the last 4 min of exercise and expressed as microvolts per heart beat (µV/bpm). From the ST/HR values we measured the difference between the ST-segment depression during and after exercise at corresponding heart rates. Since the measured value was negative in most cases, it is here referred to as ST 'deficit'. A time curve for the ST 'deficit' in each of the first 10 min of recovery was constructed.

Blood sampling

Blood samples were drawn from an arm vein (contra-lateral to the arm in which the infusion was given) at rest before start of exercise, at 2 and 4 min after start of exercise, at peak exercise, and after 4, 10 and 30 min of recovery. Plasma was separated immediately and frozen at −70 °C until analysed. NPY was assessed by radioimmunoassay. The plasma concentration of AR-H040922 was measured by liquid chromatography with fluorescence detection. The coefficients of intra-assay variation were 7.0% and 2.9% for NPY and AR-H040922, respectively. In addition routine biochemical analysis for safety reasons were performed at the pre-entry visit and at the follow-up visit 2–5 days after study day two.

Statistics

Data from two previous studies (Astra Data on file) indicate that one may expect a within-subject standard deviation of approximately 2.5 min for the time to 1 mm ST-depression. It is deemed necessary to detect a difference of 0.9 min to be able to show that the concept of the drug holds. With 56 patients it will be possible to detect such a difference with an overall power of 0.78 at an overall significance level of 0.085. This calculation assumes an interim analysis after 28 patients and is based on Student’s t-test at a significance level of 0.05, both at the interim analysis and the final analysis.

Based on the obtained within subject standard deviation for time to 1 mm ST segment depression in the first group of patients receiving the low dose of AR-H040922 of 1.5 min, another group of 24 patients were planned to be enrolled into the high dose group. This would allow detection of a difference between active treatment and placebo in mean values of 0.9 min with a power of 80% at a significant level of 5% between active treatment and placebo. Clinical measurements were analysed in a mixed ANOVA model with treatment, period, and sequence as fixed effects and patient within sequence as a random effect. Two-sided confidence intervals, with a confidence level of 95%, for the estimated mean and mean difference between values obtained during infusion of AR-H040922 and placebo, respectively, were calculated. Corresponding p-values are given. The calculations are based on Student’s t-distribution. Differences were considered significant when p<0.05, two-tailed test. Presented values are mean±SD

Results

Patients

A total number of 88 patients were included in the study. Six patients were excluded from the evaluation due to incomplete ECG recordings or due to that significant ST segment depression (>1 mm) did not occur during the study exercise. Thus, 59 patients receiving the low dose of AR-H040922 (6.7 µg/kg/min) and 23 patients receiving the high dose (13.3 µg/kg/min) were included in the final analyses of the study. Some basal characteristics of
the patients are presented in Table 1. In the following, data obtained with the two different doses are presented separately.

**Low dose AR-H040922**

Administration of AR-H040922 did not affect resting heart rate or systolic blood pressure (Fig. 1). There were no significant differences in duration of exercise or haemodynamic parameters during or after exercise between treatment with AR-H040922 and placebo (Fig. 1, Table 2). Time to >1 mm ST segment depression was 469±225 s during placebo and 458±192 s during administration of AR-H040922 (p=ns; Table 2). Maximal ST depression just before termination of exercise was unaltered by AR-H040922. The ST/HR slope, which has been shown to be a reliable index for myocardial ischaemia at the end of exercise and a reflection of the functional severity of coronary obstruction, was not changed by AR-H040922 (Table 2). Since we previously have suggested a possible involvement of NPY in myocardial ischaemia in the recovery phase after exercise, particular attention was paid to this phase. However, neither the duration of ST depression or the ST ‘deficit’ was influenced by AR-H040922 (Table 2, Fig. 2).

The plasma concentrations of AR-H040922 increased to 2948±661 nmol/l at start of exercise and to a maximum of 3569±738 nmol/l at the end of exercise. There was a positive interaction between the efficacy variables ST depression and time to

### Table 2 Exercise test variables during low dose AR-H040922

<table>
<thead>
<tr>
<th>Variable</th>
<th>AR-H040922*</th>
<th>Placebo*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to 1 mm ST depression (s)</td>
<td>458±192</td>
<td>469±225</td>
<td>ns</td>
</tr>
<tr>
<td>Duration of exercise (s)</td>
<td>723±202</td>
<td>735±215</td>
<td>ns</td>
</tr>
<tr>
<td>Duration of 1 mm ST depression (s)</td>
<td>482±318</td>
<td>483±298</td>
<td>ns</td>
</tr>
<tr>
<td>Maximal ST depression (µV)</td>
<td>204±92</td>
<td>198±98</td>
<td>ns</td>
</tr>
<tr>
<td>Duration of ST depression after exercise (s)</td>
<td>225±212</td>
<td>217±198</td>
<td>ns</td>
</tr>
<tr>
<td>ST/HR slope µV/bpm</td>
<td>−6.3±3.8</td>
<td>−5.9±3.7</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Values are mean±SD.
1 mm ST segment depression and plasma concentrations of AR-H040922. Based on these findings the study was continued in an additional group receiving a higher infusion rate of AR-H040922, 13.3 µg/kg/min.

High dose AR-H040922

Infusion of the high dose of AR-H040922 resulted in a plasma concentration of 7191±1618 nmol/l. The increase in systolic blood pressure during submaximal exercise was significantly lower during administration of AR-H040922 than during placebo (Fig. 1). Likewise, the changes in systolic blood pressure after exercise were greater with AR-H040922 than with placebo (p<0.05). HR was not affected by AR-H040922. Despite this haemodynamic effect of AR-H040922, no significant differences in time to >1 mm ST segment depression, exercise time or ECG parameters at the end of exercise were observed between active drug and placebo administration (Table 3, Fig. 2). Furthermore, when the maximal ST segment depression and the ST/HR slopes were compared at highest common workload no differences between drug treatment and placebo were observed.

NPY levels

Plasma NPY levels increased during exercise both during AR-H040922 and placebo. The increase in plasma concentrations of NPY was significantly greater during exercise with AR-H040922, regardless of dose, than with placebo (Table 4).

Side effects

Eleven patients reported some kind of side effects during the infusion. Three patients (one in the low dose group and two in the high dose group) developed hypotension during AR-H040922 requiring the infusions to be stopped. In one patient receiving the low dose, the infusion was stopped permanently in the recovery phase after exercise. In the other two patients the infusions were only stopped temporarily for 12 and 13 min, respectively. Other side effects were of non-specific character and were equally distributed between active drug and placebo.

Discussion

Since our previous study indicated that NPY might be involved as a mediator of myocardial ischaemia during exercise in patients with angina pectoris, administration of an NPY receptor antagonist was expected to be beneficial in patients with exercise-induced angina pectoris. However, the Y1 receptor antagonist AR-H040922 had no effect on exercise-induced ischaemia during or after exercise in patients with coronary artery disease. On the other hand, the Y1 receptor antagonist significantly attenuated the increase in blood pressure during exercise.

Administration of a high dose of the Y1 receptor antagonist resulted in lower systolic blood pressure both during and after exercise. This is to our knowledge the first observation suggesting involvement of endogenous NPY in the regulation of blood pressure during sympathetic activation in humans. The observation that blood pressure was lower also 30 min after cessation of the exercise in the presence of AR-H040922 suggests that NPY participates in the regulation of blood pressure for a long period after its release during sympathetic activation. This finding is in accordance with the considerable longer duration of the vasoconstrictor response evoked by NPY than by noradrenaline.

Several explanations may exist why the selective Y1 receptor antagonist did not reduce the exercise-induced ischaemia. One possibility is that NPY is not involved in the regulation of coronary artery tone and exercise-induced ischaemia. However, considering the vascular effects of NPY it is reasonable to assume that release of NPY during exercise may
Table 3 Exercise test variables during high dose AR-H040922

<table>
<thead>
<tr>
<th></th>
<th>AR-H040922*</th>
<th>Placebo*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to 1 mm ST depression (s)</td>
<td>412±146</td>
<td>408±154</td>
<td>ns</td>
</tr>
<tr>
<td>Duration of exercise (s)</td>
<td>666±190</td>
<td>644±156</td>
<td>ns</td>
</tr>
<tr>
<td>Duration of 1 mm ST depression (s)</td>
<td>395±339</td>
<td>422±297</td>
<td>ns</td>
</tr>
<tr>
<td>Duration of ST depression after exercise (s)</td>
<td>203±125</td>
<td>215±150</td>
<td>ns</td>
</tr>
<tr>
<td>ST/HR slope µV/bpm</td>
<td>−6.0±3.6</td>
<td>−5.7±3.0</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Values are mean±SD.

Table 4 Plasma levels of NPY

<table>
<thead>
<tr>
<th></th>
<th>Pre-exercise</th>
<th>Peak NPY</th>
<th>Change from pre-exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low dose AR-H040922</td>
<td>26±25</td>
<td>61±34</td>
<td>34±32*</td>
</tr>
<tr>
<td>Placebo</td>
<td>28±14</td>
<td>54±26</td>
<td>26±24</td>
</tr>
<tr>
<td>High dose AR-H040922</td>
<td>22±14</td>
<td>54±24</td>
<td>32±20*</td>
</tr>
<tr>
<td>Placebo</td>
<td>22±11</td>
<td>41±21</td>
<td>19±18</td>
</tr>
</tbody>
</table>

*P<0.05 from placebo. Values are mean±SD.

contribute to myocardial ischaemia by two different mechanisms. NPY may induce coronary constriction and thereby reducing myocardial oxygen supply. In addition, NPY may cause peripheral vasoconstriction and thereby increase afterload, which will enhance myocardial oxygen demand. Accordingly, the increase in blood pressure during exercise was significantly smaller in the presence of the high dose of AR-H040922, which may support such an effect. Exogenous NPY causes long lasting vasoconstriction in several vascular beds including the coronary circulation of both experimental animals and humans. In the coronary circulation this effect is associated with ischaemia and impaired ventricular function. Furthermore, we have previously demonstrated an association between the increase in plasma NPY and the duration of ST segment depression after exercise and the ST ‘deficit’. These observations suggest an involvement of NPY during exercise-induced myocardial ischaemia. On the other hand, when NPY was infused into the coronary arteries of patients with coronary artery disease only a subgroup of the patients (three of six) responded with coronary constriction whereas NPY was without effect in the rest of the group. Furthermore, in a recent study on healthy young volunteers i.v. administration of NPY at doses, which elevated plasma NPY levels to 3000 pmol/l, did not affect myocardial blood flow. Thus, it is possible that NPY at concentrations observed in the present study does not produce significant effects on myocardial blood flow in humans.

Another explanation may be that the effect of NPY is mediated by other receptors than Y1. There is evidence for a proportion of post-synaptic Y2 receptors (or other subtypes) since a vasoconstrictor effect has been obtained using NPY fragments specific for the Y2 receptor in certain vascular beds. The increase in systemic blood pressure and the renal and splanchnic vasoconstrictor response to NPY in humans is blocked by Y1 receptor antagonism. Furthermore, the increase in blood pressure was attenuated by Y1 receptor blockade in the present study. Thus, the Y1 receptor seems to be the dominant receptor subtype in vascular beds of haemodynamic importance.

A third possibility is that the dose of AR-H040922 was inadequate to block NPY-mediated effects. However, available data does not support this. Thus, the observation that systolic blood pressure increased less in the presence of the high dose AR-H040922 clearly suggests that this dose of the antagonist was effective. A previous study in man showed that splanchnic and renal vasoconstriction induced by exogenous NPY was blocked by the low dose of AR-H040922 used in the present study. Furthermore, it has been clearly demonstrated that the vasoconstrictor response to sympathetic nerve stimulation in the kidney and skeletal muscle of pigs is attenuated by plasma concentrations of AR-H040922 above 1000 nmol/l. Thus, the presently obtained concentrations of AR-H040922 (3000 and 7000 nmol/l) are most likely within the therapeutic range.
An observation that may influence the results of the present study is that plasma levels of NPY during exercise increased significantly more in the presence of AR-H040922 than during placebo. Similar findings have been obtained during infusion of NPY in humans. These observations may indicate that the Y1 receptor participates in the clearance of NPY from the circulation, which is in line with the role of the endothelin B receptor in the clearance of endothelin-1. Since AR-H040922 is a competitive antagonist at the Y1 receptor, the increased plasma levels of NPY in the presence of the antagonist may reverse the receptor blockade and thereby offset a therapeutic effect.

A limitation of the present protocol is that AR-H040922 was administered as a single i.v. infusion. The rationale for this protocol was the fact that AR-H040922 has poor oral bioavailability and a short plasma half life. Despite this limitation, short term administration of the high dose AR-H040922 did result in a 7 mm Hg reduction of systolic blood pressure for up to 30 min after end of exercise. It cannot be excluded, however, that prolonged and repeated administration of an orally active Y1 receptor antagonist may also have additional effects. It is not known whether ongoing medication may have interfered with the NPY receptor antagonist. The patients did not take their morning medication on the day of the exercise test. Thus, the plasma concentrations of concurrent medication are assumed to be low during the exercise tests. It has been described that metoprolol reduces the number and the affinity of NPY binding sites in vascular smooth muscle cells in hypertensive rats. It is not known, however, whether beta-blockers affect the binding of AR-H040922.

Conclusion

The present study demonstrates that selective blockade of the Y1 receptor with high dose AR-H040922 significantly inhibits the increase in blood pressure during and after exercise, indicating a role for NPY in blood pressure regulation during and following sympathetic activation in patients with coronary artery disease. However, despite this effect AR-H040922 does not influence the degree of myocardial ischemia during or after exercise.

Acknowledgements

We thank Carina Nihlén and Hanne Schulz Jensen for skilful technical assistance. The study was supported by AstraZeneca R&D Mölndal, Sweden, the Swedish Research Council (10857) and the Swedish Heart and Lung Foundation.

References


