Effects of Ifenprodil and Baclofen on Exercise-Induced Increase of Myocardial Oxygen Demand in Normotensive Rats

LAURENT MONASSIER, VÉRONIQUE RIEHL, JEAN-PAUL LIENHARD, EDUARDO TIBIRIÇA,1 JOSIANE FELDMAN, and PASCAL BOUSQUET

ABSTRACT
Central glutamatergic relays are known to be present in the central sympathetic pathways. Ifenprodil (an N-methyl-D-aspartate antagonist) and baclofen (a γ-aminobutyric acid agonist) are both modulators of these synapses; we previously reported their ability to reduce the cardiovascular responses induced by a central hypothalamic stimulation in rabbits. In this work, we investigated the actions of chronic treatments with these two drugs on the increase of myocardial oxygen demand induced by exercise in normotensive rats. Moreover, their effects on the baroreceptor heart rate reflex were observed. Male normotensive WKY rats were treated with placebo (two groups), baclofen, or ifenprodil for 14 days. They were then submitted to a progressively increased exercise test on a treadmill. In another three groups of animals, the same treatment was applied but, at the end, a baroreflex study was performed by the injection of phenylephrine (vagal component of the reflex) and of sodium nitroprusside (sympathetic component). Ifenprodil and baclofen reduced by nearly 50% the level of the increase of the rate × pressure product during exercise as compared with control rats. This effect appeared to be mainly due to a reduction of the hypertensive response. In the same conditions, neither baclofen nor ifenprodil significantly altered the baroreceptor heart rate reflex. The fact that these two drugs are capable of reducing the myocardial oxygen demand encourages us to test them in a model of myocardial ischemia associated with sympathetic hyperactivity.

It is well documented that γ-aminobutyric acid (GABA)-ergic and glutamatergic systems of neurotransmission are largely involved in the central regulation of the cardiac function (Antonaccio and Taylor, 1977; Chalmers and Pilowsky, 1991). Glutamate as well as GABA induce varied hemodynamic responses when microinjected into central brain structures involved in the regulation of blood pressure, heart rate (HR), or cardiac contractility. Such cardiovascular effects can be elicited from various areas of the forebrain (Gelsema et al., 1989; Spencer et al., 1990), of the medulla oblongata (Sun and Guyenet, 1986), and of the spinal cord (Sundaram et al., 1989). In previous studies, we described the ability of the selective GABAA agonist baclofen [β-(p-chlorophenyl)GABA] and of the N-methyl-D-aspartate (NMDA) antagonists to attenuate centrally-induced increases of myocardial oxygen consumption in the anaesthetized rabbit (Tibirica et al., 1993; Monassier et al., 1994). Ifenprodil (a NMDA receptor antagonist acting at the polyamine site) as well as baclofen (an inhibitor of presynaptic glutamate release) prevented the vascular and cardiac responses to such an activation and were devoid of marked cardiodepressive properties. These compounds could therefore be proposed as prototype drugs to treat chronic ischemic heart disease in which the increase of cardiac work induced by physical exercise is obviously deleterious. The myocardial protective effects of both drugs were so far only observed when the sympathetic activity was increased. Our previous studies were all performed in pentobarbitone-anesthetized animals to which central electrical stimulations were delivered.

In this work, we designed an experimental model of physical exercise in normotensive animals to further investigate the pharmacological profile of baclofen and ifenprodil administered in a chronic manner. In this model, the increase of myocardial oxygen demand was induced by exercise tests on a treadmill. This test was chosen because it reproduced the hemodynamic changes responsible of myocardial ischemia in patients with angina pectoris (Chierchia et al., 1990). The effects of the reference α1-adrenergic antagonist prazosin on the cardiovascular response to exercise was compared to those of ifenprodil because of well known α1-adrenergic

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ABBREVIATIONS: GABA, γ-aminobutyric acid; SAP, systolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; RPP, rate pressure product (mm Hg × beats/min × 10−3); NMDA, N-methyl-D-aspartate; SNP, sodium nitroprusside; PVN, paraventricular nucleus of the hypothalamus.
blocking properties of the latter. We have also studied the effects of baclofen and ifenprodil on the baroreflex function because glutamatergic synapses are known to be involved in this reflex and also because an alteration of the latter could avoid the use of such drugs to treat ischemic heart disease.

Materials and Methods

Exercise Testing

General Procedure. Twenty-nine normotensive male rats (WKY; Elevage Janvier, Le Genest-Saint-Ise, France), 11 weeks old at the beginning of the procedure, were divided into five groups: two placebo groups, one ifenprodil-treated group, one prazosin-treated group, and one baclofen-treated group. They were treated twice a day for 14 days, by i.p. injections of vehicle (saline 0.9%; 2 × 300 μl), ifenprodil (2 × 2.5 mg/kg), prazosin (2 × 50 μg/kg), or baclofen (2 × 5 mg/kg). The 14 control animals were randomized into two control groups. After 13 days of treatment, they were anesthetized with sodium pentobarbitone (45 mg/kg i.p.) and a polyethylene tubing catheter (PE 50: o.d. 0.96 mm; i.d. 0.58 mm) filled with heparinized saline (100 UI/ml) was inserted into the left common carotid artery. This catheter was tunneled s.c., exteriorized between the scapulae, and plugged by burning the tip. Surgery was performed using aseptic techniques and lasted about 20 min. The rats were then left to spontaneous recovery. The treatment was not disrupted during the day we performed this surgical procedure.

Exercise Test. The exercise test was performed 2 h after the surgical procedure (day 14) and 2 h after the last morning treatment dose on a motor-driven treadmill (Treadmill LI 8706; Letica, Barcelona, Spain). All of the exercise tests were performed at the same time (11:00 AM) to obtain cardiovascular responses not influenced by circadian variations of the hemodynamic status.

After 10 min of stabilization, resting data were recorded every 5 min for 15 min when the rat was standing quietly on the treadmill. Blood pressure was continuously monitored by means of the arterial catheter connected to a long human lymphographic catheter (PE 50: o.d. = 0.96 mm; i.d. = 0.58 mm) filled with heparinized saline (100 UI/ml) was inserted into the left common carotid artery. This catheter was tunneled s.c., exteriorized between the scapulae, and plugged by burning the tip. Surgery was performed using aseptic techniques and lasted about 20 min. The rats were then left to spontaneous recovery. The treatment was not disrupted during the day we performed this surgical procedure.

Baroreflex Testing. The baroreflex test was performed 2 h after the last dose of treatment (11:00 AM). For each experiment, rats were placed in a Plexiglas chamber (30 × 30 × 35 cm) and were then totally freely moving. Catheters were exteriorized from the protecting system, which was comprised of plastic tubing closed by a cap and carried by the rat by means of two plastic straps. The venous catheter was connected to a long human lymphographic catheter (lymphography catheter: model 3721; Guerbet Biomedical, Louvres, France) filled with normal saline solution to allow i.v. injections without disturbing the animal. The arterial femoral catheter was connected to the same pressure-recording system as the one described above. The two laterothoracic electrodes were plugged into the arm derivations of an electrocardiograph (EKG-Burdiick Siemens), which was in turn connected to a high-speed recorder (Minograph 803; Siemens). This procedure allowed a continuous recording of the interval between two cardiac contractions (the RR interval). The rats were given sufficient time to equilibrate to their new surroundings (±20 min), by which time they were resting quietly. Dose-response curves were constructed using i.v. bolus of phenylephrine (1, 2, 4, 8, 16, and 32 μg/kg) to increase blood pressure and of sodium nitroprusside (SNP; 1, 2, 4, 8, 16, 32, and 64 μg/kg) to decrease blood pressure. Drugs were administered in a volume of 100 μl and quickly flushed with 200 μl of vehicle (saline 0.9%). Rats were given time to recover baseline hemodynamics between two injections (nearly 5–10 min). In these rats, phenylephrine was administered first, followed by SNP 20 min after the last phenylephrine dose. HR was calculated from the RR interval corrected in function of the speed of the recorder (100 mm/s for the phenylephrine test and 250 mm/s for the SNP test).

The peak changes in MAP and the HR response to the bolus doses of phenylephrine and SNP were used for the analysis of baroreflex parameters determined by sigmoidal curve-fitting procedures. A nonlinear regression using least-squares techniques was used to reach maximum probability estimates of parameter values according to a method described extensively by others (Verberne et al., 1988; Conrad and Russ, 1992; Naoyoshi and Head, 1993). These calculations were performed using the Statview II program (Abacus Concepts, Berkeley, CA). The particular points of the curve were calculated by the mean of a computer analysis based on the equations of Widopp et al. (1990).

MAP and the HR data, forced to the resting hemodynamic conditions, were related by the mean of the following equation:

\[
HR = P1 + \text{range}/[1 + e^{A(MAP-BP50)}]
\]

where the curvature coefficient \( A = -4 \frac{G_{\text{max}}}{\text{range}} \)

\[
= -4.56 \frac{G_{\text{max}}}{\text{range}}
\]

and \( G_{\text{mean}} \), and \( G_{\text{max}} \) are, respectively, the average and the maximum gain (i.e., the slope) of the reflex calculated between the two inflection points or at the MAP value corresponding to the midpoint of the HR range (BP50). P2 and P1 are the maximum and the minimum HR plateau values of the baroreceptor curves. The baroreceptor reflex range which is P2 – P1 and the BP50 can then be calculated. TL and TU are respectively the lower and higher reflex thresholds, which represent the MAP levels at which the baroreceptors start to modify the HR and reach saturation. A correlation coefficient was calculated and indicated how the calculated curves were correlated to the experimental points.
All of the procedures described here have been carried out in accordance with the Declaration of Helsinki concerning the care and use of laboratory animals.

Drugs

The following drugs were used: heparin (Heparine Leo; Leo Laboratories, St-Quentin-en-Yvelines, France); sodium pentobarbitone (Nembutal; Abbott Laboratories, North Chicago, IL); ifenprodil (Vadilax; Synthélabo, Le Plessis-Robinson, France); d-1-baclofen, prazosin, and phenylephrine (Sigma Chemical Co., St. Louis, MO); and SNP (Niprid; Roche, Neuilly-sur-Seine, France).

Statistical Analysis

All results are expressed as means ± S.E.M. The effects of treatments on basal values from one group to the other were compared by a one-way ANOVA followed by Bonferroni’s test to determine statistically significant differences. In the exercise test protocol, the values at each step were compared by an unpaired Student’s t test (two-tailed). To analyze the effects of treatments, each treated group was compared with its own control group. Differences were considered as significant when \( P < .05 \). In the baroreflex studies, statistical differences between means of the baroreceptor HR reflex parameters (including basal parameters) were determined by a one-way ANOVA followed by Bonferroni’s test. This analysis was performed on the three groups. All calculations were made by computer-assisted analyses with the GraphPAD Instat program (version 1.11a, 1990; Amsterdam, the Netherlands).

Results

Effects of Ifenprodil

Effects of Ifenprodil on Hemodynamic Responses to Exercise. Ifenprodil given over a period of 2 weeks (5 mg/kg) twice a day did not significantly modify the basal hemodynamics. The resting mean blood pressure averaged 111 ± 6 mm Hg (\( n = 5 \)) in the ifenprodil-treated group versus 102 ± 4 mm Hg (\( n = 7 \); N.S.) in the placebo-treated one. The basal HRs were also very close: 421 ± 17 beats/min (ifenprodil-treated; \( n = 5 \)) and 424 ± 17 beats/min (controls; \( n = 7 \); N.S.). As a consequence, the basal myocardial oxygen demand index RPP was not different in the ifenprodil-treated rats from the saline-treated ones; RPP values were, respectively, 50 ± 2 and 49 ± 3.

In these two groups, exercise induced a progressive increase in blood pressure, HR, and myocardial oxygen demand (Table 1 and Fig. 1A). Nevertheless, the peak values of systolic, diastolic, and mean blood pressure were lower in ifenprodil-treated rats compared with control animals. From and after the third level of the exercise test, these values were statistically significantly different as compared with the control ones. Consequently, at these steps, the RPP response was also lowered. At the maximal effort (35 cm/s), the RPP value in the ifenprodil-treated group was 79 ± 4 (\( n = 5 \)) whereas in control animals it was 96 ± 3 (\( n = 7 \); \( P < .01 \)).

Effects of Prazosin on Hemodynamic Responses to Exercise. When compared with ifenprodil-treated animals, prazosin given 2 weeks (50 μg/kg/day) exhibited nearly the same cardiovascular profile during the exercise test (Table 2). With both drugs, the basal cardiovascular parameters were not affected. As a consequence, the resting RPPs were not significantly different in these two groups. Based on preliminary data, the dose of prazosin (50 μg/kg/day) was

![Graph showing the effects of ifenprodil on RPP](image)

**Table 1**

Effects of ifenprodil on the cardiovascular responses to a progressive exercise test in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SAP</th>
<th>DAP</th>
<th>MAP</th>
<th>HR</th>
<th>RPP</th>
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</thead>
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<tr>
<td></td>
<td>mm Hg</td>
<td>mm Hg</td>
<td>mm Hg</td>
<td>beats/min</td>
<td>mm Hg</td>
</tr>
<tr>
<td>Groups</td>
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<td>Ifen</td>
<td>Placebo</td>
<td>Ifen</td>
<td>Placebo</td>
</tr>
<tr>
<td>Rest</td>
<td>115 ± 4</td>
<td>120 ± 5</td>
<td>95 ± 4</td>
<td>105 ± 5</td>
<td>102 ± 4</td>
</tr>
<tr>
<td>Level 1 (5 cm/s)</td>
<td>147 ± 7</td>
<td>134 ± 6</td>
<td>129 ± 5</td>
<td>121 ± 8</td>
<td>138 ± 6</td>
</tr>
<tr>
<td>Level 2 (15 cm/s)</td>
<td>156 ± 5</td>
<td>138 ± 7</td>
<td>142 ± 5</td>
<td>122 ± 10</td>
<td>147 ± 5</td>
</tr>
<tr>
<td>Level 3 (25 cm/s)</td>
<td>165 ± 4</td>
<td>140 ± 6*</td>
<td>152 ± 4</td>
<td>122 ± 9*</td>
<td>157 ± 4</td>
</tr>
<tr>
<td>Level 4 (35 cm/s)</td>
<td>172 ± 4</td>
<td>149 ± 6*</td>
<td>155 ± 4</td>
<td>121 ± 12*</td>
<td>160 ± 4</td>
</tr>
</tbody>
</table>

* \( P < .05 \); ** \( P < .01 \); comparison between ifenprodil-treated animals and control animals at rest and at each level of the test (two-tailed unpaired Student’s t test for comparisons).
chosen to induce no hypotension. In this case, because it was the maximal dose devoid of hypotensive effect, ifenprodil and prazosin could be compared. Increases in systolic blood pressure, HR, and of RPP were parallel in rats treated with ifenprodil or with prazosin; differences never reached statistical significance. The RPPs at the last step of the test were, respectively, 79 ± 4 and 74 ± 4 (n = 5 versus n = 5, P > .05).

**Effects of Ifenprodil on Resting Parameters and on Baroreceptor Reflex Activity.** In an other series of six animals, ifenprodil was given twice a day (5 mg/kg/day) over a period of 2 weeks. Again, no variation of the basal blood pressure was observed in comparison with controls. Resting MAP was 90 ± 4 mm Hg (n = 6) in ifenprodil-treated animals versus 94 ± 3 mm Hg in control animals (n = 6; N.S.). In contrast, in ifenprodil-treated animals, the basal HR was increased from 399 ± 6 to 465 ± 17 beats/min (P < .05; Table 3 and Fig. 2).

The mean baroreceptor HR reflex curves obtained in ifenprodil-treated and control animals derived from individual lines of best fit were not significantly different. The sympathetic arm of the reflex loops were nearly superimposed as shown by P2 values, which were 544 ± 28 beats/min in controls versus 542 ± 16 beats/min in ifenprodil-treated animals (n = 6 in each group; N.S.) and by TL parameters that were 93 ± 3 and 98 ± 5, respectively (N.S.). Neither the sensitivity nor the gain was modified as $G_{\text{max}}$ was $-6.0 \pm 0.8$ beats/min/mm Hg in controls and $-6.7 \pm 0.7$ in ifenprodil-treated animals (N.S.). Compared with control animals, ifenprodil did not alter the vagoal component of the baroreflex up to 150 mm Hg. At higher blood pressure levels, this drug surprisingly provoked a slight potentiation of the bradycardia; this did not reach statistical significance.

**TABLE 3**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Placebo (n = 6)</th>
<th>Ifenprodil (n = 6)</th>
<th>Baclofen (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal MAP (mm Hg)</td>
<td>94 ± 3</td>
<td>90 ± 4</td>
<td>95 ± 7</td>
</tr>
<tr>
<td>Basal HR (beats/min)</td>
<td>399 ± 16</td>
<td>465 ± 17*</td>
<td>457 ± 17*</td>
</tr>
<tr>
<td>$G_{\text{max}}$ (beats/min/mm Hg)</td>
<td>$-6.0 \pm 0.8$</td>
<td>$-6.7 \pm 0.7$</td>
<td>$-6.6 \pm 0.6$</td>
</tr>
<tr>
<td>$G_{\text{max}}$ (beats/min/mm Hg)</td>
<td>$-5.5 \pm 0.9$</td>
<td>$-5.5 \pm 0.7$</td>
<td>$-5.6 \pm 0.6$</td>
</tr>
<tr>
<td>P1 (beats/min)</td>
<td>162 ± 28</td>
<td>143 ± 21</td>
<td>202 ± 19</td>
</tr>
<tr>
<td>P2 (beats/min)</td>
<td>544 ± 28</td>
<td>542 ± 16</td>
<td>536 ± 21</td>
</tr>
<tr>
<td>Range (beats/min)</td>
<td>382 ± 27</td>
<td>399 ± 24</td>
<td>333 ± 25</td>
</tr>
<tr>
<td>BP50 (mm Hg)</td>
<td>115 ± 3</td>
<td>120 ± 7</td>
<td>124 ± 8</td>
</tr>
<tr>
<td>TU (mm Hg)</td>
<td>137 ± 6</td>
<td>141 ± 9</td>
<td>141 ± 8</td>
</tr>
<tr>
<td>TL (mm Hg)</td>
<td>93 ± 3</td>
<td>98 ± 5</td>
<td>106 ± 8</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.98 ± 0.01</td>
<td>0.95 ± 0.01</td>
<td>0.86 ± 0.01</td>
</tr>
</tbody>
</table>

Resting parameters: a resting tachycardia was observed in rats treated by both ifenprodil and baclofen (P < .05). Baroreflex: the parameters are means ± S.E.M. derived from individual curves fitted to the data obtained from all rats in each group, studied at the 14th day of treatment. No significant difference was observed between the three groups.

**Fig. 2.** Mean baroreceptor HR reflex curves in control (n = 6), ifenprodil (5 mg/kg/day; n = 6), and baclofen (10 mg/kg/day; n = 6) treated rats. Each curve was derived from individual lines of best fit. There was no statistical difference between those three curves (one-way ANOVA for testing differences).

**Effects of Baclofen on Hemodynamic Responses to Exercise.** Baclofen (10 mg/kg/day) given twice a day over a period of 2 weeks did not significantly affect basal hemodynamic parameters. The resting mean blood pressure averaged 111 ± 7 mm Hg (n = 5) in the baclofen-treated group when it was 103 ± 4 mm Hg (n = 7; N.S.) in the placebo-treated control animals. The basal HRs were not different either: 413 ± 26 and 395 ± 12 beats/min (n = 5 versus n = 7; N.S.) respectively. As a consequence, the resting myocardial oxygen-demand index RPP was not significantly modified by baclofen as compared with saline-treated animals; RPP values were, respectively, 51 ± 4 and 45 ± 3 (N.S.; Table 4 and Fig. 1B).

In these two groups, exercise induced a progressive increase in blood pressure, HR, and myocardial oxygen demand but the hemodynamic responses to exercise were attenuated in baclofen-treated animals. Baclofen decreased the peak values of systolic, diastolic, and mean blood pressure as compared with control animals. From the third level of the exercise test, these values were significantly different from the control ones. Consequently, at the higher steps, the RPP response was also lowered. At the highest speed (35 cm/s), the RPP value in the baclofen-treated group was 74 ± 5 (n = 5) as compared with 98 ± 2 in control animals (n = 7; P < .01).

**Effects of Baclofen on Resting Parameters and on Baroreceptor Reflex Activity.** Chronic administration of baclofen (10 mg/kg/day) did not affect resting blood pressure as compared with controls. Resting MAP was 95 ± 7 mm Hg (n = 5) in baclofen treated rats when it was 94 ± 3 mm Hg in controls (n = 6; N.S.). In contrast, like in ifenprodil-treated
rats, a basal tachycardia was observed in baclofen-treated animals. Their basal HR was 457 ± 17 beats/min, the one obtained in control animals being 399 ± 16 (P < .05; Table 3 and Fig. 2).

The mean baroreceptor HR reflex curves obtained in baclofen-treated and control animals derived from individual lines of best fit were not significantly different. The sympathetic arm of the reflex loops were similar as shown by P2 values, which were 544 ± 8 and 514 ± 21 beats/min in baclofen-treated ones (n = 5 versus n = 6; N.S.) and by TL parameters that were 106 ± 8 and 98 ± 5, respectively (N.S.). Neither the sensitivity nor the gain of the reflex were modified as Gmax was not statistically significant (Table 3 and Fig. 2).

### Discussion

In the present study, we showed that ifenprodil and baclofen both attenuated the increase of myocardial oxygen demand index RPP during exercise, without alteration of the baroreflex reactivity.

During exercise, the increase of myocardial oxygen consumption is a consequence of the tachycardia as well as of the raised cardiac contractility and arterial blood pressure (Robinson, 1967; Laslett et al., 1985). This typical hemodynamic response during exercise is due to a very fast neurohumoral adaptation in which the sympathetic nervous system plays a key role, as reflected by the increase of the plasma catecholamines in the coronary sinus during physical effort (Cousineau et al., 1977; Watson et al., 1980). Thus drugs that could smooth sympathetic hyperactivity could also diminish the myocardial oxygen consumption during physical effort. This clearly holds true with β-blocking drugs but with depressive consequences on resting cardiac function, i.e., bradycardia and reduced cardiac contractility. Aiming to study the potential cardioprotective properties of centrally acting non cardiodepressive drugs, we previously developed an experimental model in anesthetized rabbits (Tibiriça et al., 1993; Monassier et al., 1994). In this model, myocardial oxygen demand was increased by central sympathetic stimulation. In fact, the electrical stimulation of the paraventricular nucleus of the hypothalamus (PVN), which is an integrative area in the cardiovascular regulation (Hosoya et al., 1991), raised the arterial blood pressure and the cardiac contractility. These responses were prevented by modulators of central glutamatergic pathways that are known to be involved in the control of the sympathetic drive to the heart and vessels (Chalmers and Pilowsky, 1991). A variety of pre- or postsynaptic acting substances are known to modulate the glutamatergic transmission. In a previous report, we showed that blockers of the NMDA type of ionotropic glutamate receptors (see McBain and Mayer, 1994, for a review), including the NMDA polyamine site antagonist, ifenprodil (Carter et al., 1989) blunted the hemodynamic responses to PVN electrical stimulation in rabbits (Monassier et al., 1994). Moreover the GABA<sub>B</sub> agonist, baclofen, known to presynaptically inhibit glutamate release (Davies, 1981), also reduced the PVN stimulation-induced hemodynamic responses (Tibiriça et al., 1993). Nevertheless, all these effects were up to now observed in acute experiments performed in anesthetized animals.

In the present work, ifenprodil and baclofen were selected because of their efficiency in the PVN stimulation model, and their good acceptability in patients. Here, we studied the actions of long-term treatments in awake animals on the hemodynamic responses to a physiologic stimulus: the exercise test on the treadmill. The dose of baclofen was selected considering our previous results obtained with subchronic treatments in rabbits, showing that the 5 mg/kg/day dose inhibited the centrally induced increase of sympathetic nervous system activity. The dose of ifenprodil was selected in preliminary experiments as the highest dose, which did not reduce arterial blood pressure. Ifenprodil and baclofen were both capable in reducing the pressor response to physical exercise. A tendency to a reduction of the tachycardic response was also observed. According to our general experience, rats with carotid artery ligation exhibited resting tachycardia and an increase in blood pressure. This might be due to the carotid artery ligation itself as a consequence of a slight inhibition of the baroreflex induced by this procedure. However, these weak variations of the basal hemodynamics did not compromise our experimental procedure. The myocardial oxygen demand index, RPP, was markedly increased by exercise in control animals (about 100%). In animals treated with both glutamatergic synapse modulators, RPP still increased but to a lesser extent. The reduction of the afterload response (blood pressure) during exercise was mainly responsible for the reduction of the RPP. Although not statistically significant, the slight drug-induced reduction of the tachycardic response also contributed to the decrease of the myocardial oxygen demand during exercise. Although myocardial contractility was not measured in this

### Table 4

Effects of baclofen on the cardiovascular responses to a progressive exercise test in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SAP</th>
<th>DAP</th>
<th>MAP</th>
<th>HR</th>
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<tr>
<td></td>
<td>mm Hg</td>
<td>mm Hg</td>
<td>mm Hg</td>
<td>beats/min</td>
<td>mm Hg</td>
</tr>
<tr>
<td>Groups</td>
<td>Placebo</td>
<td>Baclo</td>
<td>Placebo</td>
<td>Baclo</td>
<td>Placebo</td>
</tr>
<tr>
<td>Rest</td>
<td>114 ± 4</td>
<td>125 ± 7</td>
<td>97 ± 5</td>
<td>104 ± 7</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>Level 1 (5 cm/s)</td>
<td>143 ± 3</td>
<td>140 ± 9</td>
<td>127 ± 2</td>
<td>125 ± 10</td>
<td>134 ± 3</td>
</tr>
<tr>
<td>Level 2 (15 cm/s)</td>
<td>153 ± 4</td>
<td>141 ± 9</td>
<td>139 ± 5</td>
<td>125 ± 10</td>
<td>147 ± 6</td>
</tr>
<tr>
<td>Level 3 (25 cm/s)</td>
<td>168 ± 4</td>
<td>141 ± 9*</td>
<td>150 ± 7</td>
<td>126 ± 8*</td>
<td>156 ± 6</td>
</tr>
<tr>
<td>Level 4 (35 cm/s)</td>
<td>175 ± 4</td>
<td>137 ± 8*</td>
<td>155 ± 7</td>
<td>123 ± 8*</td>
<td>162 ± 6</td>
</tr>
</tbody>
</table>

*P < .05, †P < .01: comparison between baclofen-treated animals and control animals at rest and at each level of the test (two-tailed unpaired Student’s t test for comparisons).
study, it was shown to be reduced by baclofen and ifenprodil in previous experiments performed in rabbits submitted to central sympathetic stimulation (Tibiriça et al., 1993; Monassier et al., 1994). Whether these effects have a central origin is currently under investigation. A peripheral site of action of baclofen seems unlikely, as all of its known cardiovascular effects in intact animals are known to be mediated by central GABA<sub>B</sub> receptors (Hong and Henry, 1991). However, the site of action of ifenprodil remains unclear. This drug is a hybrid one, displaying polyamine NMDA receptor and α<sub>1</sub>-adrenergic antagonist properties (Chenard et al., 1991). Moreover, a rather high affinity for σ-opiate receptors was reported (Karbon et al., 1990). At the doses used here, neither ifenprodil nor prazosin decreased blood pressure. Thus, in both cases, an important vascular α<sub>1</sub>-blocking effect seems unlikely. Nevertheless, in baroreflex studies, a rightward shift of the dose-response curve to low doses of phenylephrine was observed in rats treated with ifenprodil (data not shown). Therefore, an α<sub>1</sub>-adrenergic blocking effect in vessels might account for the similar diminished vascular responses to exercise in rats treated with ifenprodil and with prazosin. Nevertheless, a central sympatholytic action mediated by a central α<sub>1</sub>-adrenergic blocking effect cannot be ruled out with these two drugs because both of them are known to cross the blood-brain barrier (Knutsson et al., 1974; Ebert et al., 1997). This has been clearly demonstrated with prazosin which can, by a true centrally mediated effect, reduce the peripheral consequences of a central nervous system activation. This was obtained after i.v. doses lower than those required to block vascular α<sub>1</sub>-adrenergic receptors (Ito et al., 1988; Koss, 1992). In our experimental conditions, the effects of ifenprodil during exercise could be mediated by a central or a peripheral α<sub>1</sub>-adrenergic blocking action, or more likely by both. The involvement of σ-receptors is also possible, although no role of σ-receptors has been described yet in the cardiovascular regulation, except in the regulation of calcium influx in cultured myocardial cells (Novakova et al., 1995). Moreover, it is noteworthy that σ-receptors are closely related to the NMDA receptor (Paul et al., 1990) and that σ-receptor ligands can also reduce glutamate release (Ellis and Davies, 1994). Thus, the mechanisms of the effects described here might be complex: α<sub>1</sub>-adrenergic antagonism (peripheral or central or both), polyamine site NMDA receptor blockade, or a σ-receptor modulation. The definitive identification of the pharmacological mechanism of action of ifenprodil in this model needs further investigation. For this purpose, selective polyamine site antagonists derived from ifenprodil will be useful.

In the second part of this work, we investigated the possible effects of these drugs on the baroreceptor HR reflex. Numerous studies reported that glutamate receptor agonists and antagonists affect the baroreflex when applied to various cerebral structures such as the nucleus of the tractus solitarius (Guyenet et al., 1987), the rostroventrolateral medulla (Sun and Guyenet, 1987), or into different nuclei of the hypothalamus (Spencer et al., 1990). Moreover, glutamatergic relays are involved in the central control of preganglionic neurons in the efferent sympathetic pathways to the heart and vessels (Sundaram et al., 1989). Baclofen and ifenprodil are able to provoke orthostatic hypotension at the beginning of treatments in patients, probably because of a reduced efficiency of the sympathetic component of the baroreflex loop. In this part of the study, ifenprodil and baclofen were given to animals with intact carotid arteries. In our experimental model, neither ifenprodil nor baclofen applied chronically altered the baroreflex sympathetic HR activation. This could be explained either by a baroreflex resetting counteracting an inhibitory effect of the drugs or to a real absence of significant influence of these drugs on the baroreflex sympathetic reactivity. Moreover, the baroreflex reactivity to low pressures seems to be different from the one to high pressures. Compared with control animals, neither of these two drugs altered the vagal component of the baroreflex up to 150 mm Hg. At higher blood pressure levels, we observed a difference between ifenprodil and baclofen responses. Baclofen slightly lowered the maximal bradycardia compared with that observed in control animals. This could be due to a weak inhibition of the nucleus of the tractus solitarius as previously observed after microinjection of this drug into this structure (Bousquet et al., 1982). Unlike other NMDA antagonists, ifenprodil surprisingly provoked a slight potentiation of the bradycardia. Such a phenomenon has been described previously in an experimental model of cerebral ischemia in anesthetized dogs in which ifenprodil restored the baroreflex sensitivity altered by ischemia (Kurihara et al., 1990). For the authors of this work, this phenomenon was a consequence of a neuroprotective effect of ifenprodil, but it could be interpreted as a direct baroreflex vagal stimulation. Microinjections of NMDA antagonists have been described as inhibitors (Kubo and Kihara, 1991) or activators (Li and Blessing, 1990; Masuda et al., 1991) of central structures involved in the vagal component of the baroreflex loop in anesthetized animals. Moreover, Sved and Tsukamoto (1992) have described an inhibitory effect of baclofen microinjected into the nucleus tractus solitarii on the baroreflex response to baroreceptor afference stimulation. Thus, it seems that the mechanism of the baroreflex effect (inhibition or activation) depends on the site of injection. In our conditions of chronic systemic administration, we probably observed an overall effect resulting from conflicting influences (originating in different brain structures) that roughly compensate each other.

Finally, baclofen and ifenprodil reduced myocardial oxygen demand during exercise without marked alteration of the resting hemodynamics and baroreceptor HR reflex. An ischemic action of these drugs now needs to be confirmed in animals with myocardial ischemia.

References


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