Abstract We investigated the role of nitric oxide on rapid (25- and 40-minute) baroreceptor resetting during the onset of acute hypertension in rats treated with \(N^0\)-nitro-L-arginine, an inhibitor of nitric oxide synthesis, and methylene blue, an inhibitor of guanylate cyclase. The effect of treatment with glibenclamide, an ATP-dependent \(K^+\) channel blocker, was also investigated. Arterial hypertension was provoked in a ramp progression by the drug \(N^0\)-nitro-L-arginine alone or in association with aortic coarctation. Whole aortic nerve activity and carotid pressure were recorded in the anesthetized rats. The extent of rapid resetting was evaluated by means of the ratio (\(\Delta\)Systolic Threshold Pressure/\(\Delta\)Control Diastolic Pressure)\(\times 100\) as well as by the extent of displacement of the pressure–nerve activity curve defined by the ratio (\(\Delta\)Mean Arterial Pressure at 50% of maximum activity/\(\Delta\)Mean Arterial Pressure)\(\times 100\). All groups gave the same increase in mean arterial pressure at 25 and 40 minutes after the onset of hypertension. A greater extent of resetting to hypertensive levels was observed in the treated groups compared with coarctation alone. At 40 minutes after the onset of hypertension, the coarctation and \(N^0\)-nitro-L-arginine groups exhibited a further increase in the extent of resetting. The rats submitted to glibenclamide plus coarctation presented a slight but significant decrease in gain. These findings suggest that an active \(L^--\)arginine–nitric oxide–cyclic GMP pathway blunts rapid resetting during the onset of hypertension. In addition, they also indicate that ATP-dependent \(K^+\) channels can also modulate rapid resetting of the baroreceptors to hypertensive levels.

Key Words • glibenclamide • guanosine cyclic monophosphate • nitric oxide • methylene blue • pressoreceptors • \(N^0\)-nitro-L-arginine

Acute (or rapid) resetting of the baroreceptor occurs within seconds to minutes after sustained changes in conditioning pressure. When changes in pressure are sustained for several minutes, there is a decay in activity at constant pressure and a rightward shift in the pressure–nerve activity relation. This phenomenon has also been demonstrated to occur in the opposite direction after the exposure of the baroreceptors to hypotension. Rapid resetting is considered a partial process that exhibits no change in gain (slope of the pressure–nerve activity curve). Nevertheless, when rapid resetting was driven to hypotensive levels under the influence of various antihypertensive agents, the baroreceptors reset totally within 15 minutes to hypotensive levels, even though each of the vasodilators (sodium nitroprusside, nifedipine, and captopril) had different primary mechanisms of action. Therefore, the mechanism by which the baroreceptors reset rapidly and totally still remains obscure. Changes in the mechanical properties of the vessel wall, ionic transport across the membrane of the baroreceptor endings, and substances released from the endothelium are important candidate mechanisms to explain rapid resetting. Inhibitory or excitatory substances may be released by the endothelium during vascular distension, after arterial pressure increase, and may modulate the extent of rapid resetting. Recent findings demonstrated that nitric oxide (NO) and nitrosocysteine decrease baroreceptor activity by means of a cyclic GMP (cGMP)–independent mechanism. The synthase inhibitor, \(N^0\)-nitro-L-arginine (LNA) is an inhibitor of NO synthesis that causes by itself a slow increase in ramp form in arterial pressure when infused intravenously. On the other hand, methylene blue (MB) is an effective inhibitor of guanylate cyclase, which does not significantly affect the basal arterial pressure. Therefore, we investigated the effect of blockers (LNA and MB) of the \(L^--\)arginine–NO–cGMP pathway on rapid (30-minute) baroreceptor resetting during the onset of hypertension caused by the intrinsic effect of LNA or by controlled aortic coarctation (COA) associated with MB. In addition, because solutions with low \(K^+\) blunted rapid (5- to 15-minute) resetting to hypertensive levels, we also investigated the role played by ATP-dependent \(K^+\) channels on rapid resetting by means of glibenclamide, an ATP-dependent \(K^+\) channel blocker.

Methods

Experiments were performed on anesthetized (thiopental sodium, 40 mg/kg IP) normotensive male Wistar rats (250 to 300 g) in accordance with the guiding principles of the American Physiological Society. Catheters were placed into the right common carotid artery for measurement of arterial pressure, the right femoral vein for the administration of drugs. A pneumatic cuff was placed around the abdominal aorta immediately below the diaphragm to produce an acute (25- to 40-minute) hypertensive response in ramp.

The procedure for recording the whole-nerve activity of the aortic baroreceptor was the same as that used in previous studies with some modifications. Briefly, the left aortic nerve was isolated and supported by a bipolar stainless steel electrode.
Changes in Mean Arterial Pressure, Extent of Resetting of Baroreceptors, and Extent of Displacement of Pressure–Nerve Activity Curve 25 and 40 Minutes After Ramp Increases in Arterial Pressure Elicited by Aortic Coarctation, N°-Nitro-L-Arginine, and Coarctation Associated With Methylen Blue or Glibenclamide

<table>
<thead>
<tr>
<th>Group</th>
<th>25 Min</th>
<th>40 Min</th>
<th>25 Min</th>
<th>40 Min</th>
<th>25 Min</th>
<th>40 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>COA</td>
<td>38±3</td>
<td>38±3</td>
<td>23±5</td>
<td>40±4*</td>
<td>40±4</td>
<td>52±5</td>
</tr>
<tr>
<td>LNA</td>
<td>33±3</td>
<td>34±5</td>
<td>44±6†</td>
<td>64±9‡</td>
<td>73±11</td>
<td>92±15*</td>
</tr>
<tr>
<td>MB+COA</td>
<td>44±5</td>
<td>43±3</td>
<td>45±6†</td>
<td>50±6</td>
<td>53±10</td>
<td>56±10</td>
</tr>
<tr>
<td>GLIB+COA</td>
<td>45±5</td>
<td>44±5</td>
<td>45±4†</td>
<td>46±5</td>
<td>80±11†</td>
<td>78±10</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; EOR, extent of resetting; EOD, extent of displacement; COA, aortic coarctation; LNA, N°-nitro-L-arginine; MB, methylene blue; and GLIB, glibenclamide.

*P<.05 compared with 25-minute period in the same group.
†P<.05 compared with COA in the same period.
‡P<.05 compared with COA in the same period.

and carefully insulated with silicone rubber (Wacker sil gel 604, Wacker Co, Munich, FRG). Carotid pressure was recorded simultaneously with aortic nerve discharges on an oscilloscope (model 5113, Tektronix, Beaverton, Ore) and monitored with a loudspeaker. A nerve traffic analyzer (model 605C, University of Iowa Bioengineering, Iowa City) counted action potentials that exceeded a selected voltage. Integrated aortic nerve activity and carotid pressure were recorded on a two-channel chart recorder (Narcotrace 40, Narco Biosystems, Houston, Tex). For the determination of baroreceptor firing range, rats were submitted to rapid (20- to 30-second) changes in arterial pressure by withdrawal and reinfusion of blood into the femoral artery. To avoid the influence of histeresis, we used only the values obtained during reinfusion of blood, when pressure increased (rate approximately 2 to 3 mm Hg/s). The systolic threshold pressure (SPth) for baroreceptor activation and the pressure–nerve activity curve were determined. Aortic nerve activity (spikes per second) was analyzed in relative units (percent of maximum activity). Normalized baroreceptor activity was plotted against mean arterial pressure (MAP) to obtain pressure–nerve activity curves. The pressure at 50% of maximal activity (MAP50) and the baroreceptor gain (slope of the linear portion of the curves) were calculated. The indexes used to evaluate the extent of rapid resetting of the baroreceptors were (ASPH/ACDP)×100, where CDP is control diastolic pressure, and (AMAP/ΔMAP)×100, which defines the shift of the entire pressure–nerve activity curve during rapid resetting.

Experimental Protocol

Protocol 1: N°-nitro-L-arginine

After the recording of normal baroreceptor activity in normotensive animals, LNA (15 mg/kg IV) was slowly injected into the right femoral vein during 2 minutes. LNA produced by itself a hypertensive response in ramp form at a speed of 1.5 mm Hg/min, attaining a peak value approximately 25 minutes later and remaining at this hypertensive level throughout the experiment. Baroreceptor activity was then recorded at the peak of the hypertensive response and 15 minutes later.

Protocol 2: Methylen Blue or Glibenclamide Plus Aortic Coarctation

Normotensive rats received MB (2.2 mg/kg per minute IV) or glibenclamide (2 mg/kg IV bolus). MB was dissolved in saline and infused at a rate of 0.07 mL/min, and glibenclamide was prepared by dissolving 0.6 mg/0.2 mL dimethyl sulfoxide into 3.8 mL of physiological saline solution immediately before use. The arterial pressure was then increased slowly (ramp) by progressive aortic constriction. The rate of increase in pressure was equivalent for both groups. The speed (1.5 mm Hg/min) was the same provoked by the hypertensive effect of LNA itself. Recording of the baroreceptor activity curve was performed approximately 25 minutes after the onset of hypertension and again after 15 minutes of sustained hypertension.

A group of control rats was submitted to the same protocol. They were exposed to COA hypertension without receiving any drug.

Data Analysis

Baroreceptor activity is expressed as a percent of maximum activity obtained during the increase in pressure. The slope of the linear portion of the pressure–nerve activity relation over the pressure range of 80 to 150 mm Hg was calculated with linear regression analysis. Resetting was considered partial (not complete) when the magnitude of the shift in SPth (or MAP50) differed significantly from the magnitude of the change in CDP (or MAP); otherwise, resetting was considered total (complete). Results are presented as mean±SEM. The Student’s paired t test was used to determine whether the extent of resetting within each group was partial or total. Slope and maximum baroreceptor activities were compared among groups with one-way analysis of variance and the Student’s unpaired t test. Analysis of variance with Bonferroni’s multiple comparisons test was used to compare the effects of changes in holding pressure within each group. Changes were considered significant at a value of P<.05.

Results

Changes in MAP, extent of resetting, and extent of displacement 25 and 40 minutes after the onset of hypertension induced by LNA or COA associated or not with MB or glibenclamide are shown in the Table. All groups exhibited a similar increase in MAP and a partial rapid baroreceptor resetting (Table). Compared with COA alone, LNA, MB plus COA, or glibenclamide plus COA facilitated rapid resetting. At 40 minutes, COA alone and LNA provoked a further increase in the extent of resetting compared with 25 minutes of arterial pressure elevation.

The effect of a hypertensive stimulus caused by these same treatments on pressure–nerve activity is shown in the Figure. The same increase in MAP produced in all groups a rightward shift of the pressure–nerve activity curve 25 and 40 minutes after the onset of hypertension without a change in gain (slope), except for rats submitted to glibenclamide plus COA. This group presented a slight but significant decrease in the slope of the curve at 25 minutes. The extent of displacement (Table) of the pressure–nerve activity curve of rats submitted to MB
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Discussion

We investigated the modulation of rapid resetting of the baroreceptors of normotensive rats to an increase in ramp in MAP. The hypertensive stimulus elicited a greater extent of resetting under the influence of LNA, MB, and glibenclamide compared with COA alone at 25 minutes of the onset of hypertension. Only LNA produced a further increase in the extent of resetting at 40 minutes of arterial pressure elevation. Rapid resetting has been referred to as a partial process. Nevertheless, data from our laboratory have demonstrated that various antihypertensive drugs (sodium nitroprusside, nifedipine, and captopril) allow a complete rapid resetting to hypotensive levels within 15 minutes. The mechanism responsible for the facilitation of rapid resetting by antihypertensive drugs is not yet clear. On the other hand, vascular stretch due to a hypertensive stimulus is able to stimulate the endothelium to release a number of substances, particularly the endothelium-derived relaxing factor, which may affect baroreceptor function, as seen in the present study. We found that the NO synthase inhibitor LNA caused an increase in MAP, associated with a greater extent of rapid resetting, compared with that caused by COA alone. This suggests that NO, or a related compound derived from L-arginine, affects baroreceptor resetting. NO activates soluble guanylate cyclase in several tissues, including vascular smooth muscle cells and neurons. Because MB, a blocker of guanylate cyclase activation by NO, also facilitated rapid resetting, it is possible that NO interferes with the resetting of the baroreceptors through the activation of guanylate cyclase.

Baroreceptor resetting provoked by changes in mechanical properties of the vessel wall may alter the tension of the receptors; however, ionic mechanisms operating at the neuronal membrane may also modulate baroreceptor sensitivity. In the present work we also investigated whether changes in K⁺ conductances contribute to rapid resetting, elicited by a gradually applied and then sustained hypertensive stimulus. The ATP-dependent K⁺ channel blocker glibenclamide also facilitated rapid resetting evidenced by an increase in both the extent of resetting and the extent of displacement of the pressure–nerve activity curve. Therefore, these findings suggest that ATP-dependent K⁺ channels in the open state blunt baroreceptor resetting.

Whether the observed facilitation of baroreceptor resetting caused by LNA, MB, or glibenclamide results from an indirect effect of these drugs on vessel wall mechanics or from a direct action on the baroreceptors remains to be determined. In conclusion, our data indicate that the L-arginine–NO–cGMP pathway blunts rapid resetting to hypertensive levels. In addition, ATP-dependent K⁺ channels also seem to be involved in the modulation of rapid resetting of the baroreceptors to hypertension.

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