β-Adrenoceptor Dysfunction After Inhibition of NO Synthesis

Erin J. Whalen, Alan Kim Johnson, Stephen J. Lewis

Abstract—G, protein–coupled β-adrenoceptors rapidly desensitize on exposure to agonists in reconstituted membrane preparations, whereas rapid tachyphylaxis to β-adrenoceptor–mediated vasodilation does not readily occur in vivo. This study examined the possibility that endothelium-derived nitrosyl factors prevent the rapid desensitization of β-adrenoceptors in the vascular smooth muscle of resistance arteries in pentobarbital-anesthetized rats. The fall in mean arterial blood pressure and in hindquarter vascular resistance produced by the β-adrenoceptor agonist isoproterenol (ISO, 0.1 to 10 μg/kg IV) was slightly but significantly smaller in rats treated with the NO synthase inhibitor N\textsuperscript{\textomega}-nitro-L-arginine methyl ester (L-NAME, 100 μmol/kg IV) than in saline-treated rats. The ISO-induced fall in mesenteric resistance was similar in L-NAME–treated and in saline-treated rats. The fall in hindquarter vascular resistance and in mesenteric resistance produced by ISO (8×10 μg/kg IV) was subject to tachyphylaxis on repeated injection in rats treated with L-NAME (100 μmol/kg IV) but not in rats treated with saline. Injections of L-S-nitrosocysteine (1200 nmol/kg IV), a lipophobic \textit{S}-nitrosothiol, before each injection of ISO (10 μg/kg IV) prevented tachyphylaxis to ISO in L-NAME–treated rats. The vasodilator effects of ISO (0.1 to 10 μg/kg IV) in L-NAME–treated rats that received 8 injections of ISO (10 μg/kg IV) were markedly smaller than in L-NAME–treated rats that received 8 injections of saline. These results indicate that (1) the vasodilator actions of ISO in pentobarbital-anesthetized rats only minimally involve the release of endothelium-derived nitrosyl factors, (2) the effects of ISO are subject to development of tachyphylaxis in L-NAME–treated rats, and (3) tachyphylaxis to ISO is prevented by L-S-nitrosocysteine. These findings suggest that endothelium-derived nitrosyl factors may prevent desensitization of β-adrenoceptors in vivo. (Hypertension. 2000;36:376-382.)

Key Words: adrenergic receptor agonists • hemodynamics • nitric oxide • rats

On exposure to catecholamines or the β-adrenoceptor (β-AR) agonist isoproterenol (ISO), G\textsubscript{i} protein–coupled β-ARs are rapidly (in minutes) desensitized in isolated cells and in reconstituted receptor–G\textsubscript{i} protein preparations.\textsuperscript{1–4} Rapid desensitization involves phosphorylation of β-ARs by cAMP-dependent protein kinase and by G protein–coupled receptor kinases, known as β-AR kinases.\textsuperscript{2–4} Phosphorylated β-ARs cannot be coupled with G\textsubscript{i} proteins and are sequestered into intracellular organelles, where they are dephosphorylated before reincorporation into plasma membranes.\textsuperscript{2–4} In contrast, the vasodilation produced by ISO in human forearm arteries does not diminish during a 4-hour infusion.\textsuperscript{5} These findings suggest that (1) rapid desensitization of β-ARs in vivo is prevented by endogenous factors not present in isolated cells or in reconstituted β-AR–G\textsubscript{i} protein preparations,\textsuperscript{1–4} and/or (2) the absence of these factors precludes resensitization of β-ARs.

On the basis of findings with pituitary adenylate cyclase–activating polypeptide-27, a G\textsubscript{i} protein–coupled receptor agonist,\textsuperscript{6–9} the hypothesis underlying the present study is that endothelium-derived NO–containing factors (NOFs), such as L-S-nitrosocysteine (L-SNC),\textsuperscript{9–11} prevent rapid desensitization of β-ARs in vascular smooth muscle in resistance vessels in vivo by mechanisms other than the generation of cGMP. The main goal of the present study was to determine whether ISO-mediated vasodilation is subject to rapid tachyphylaxis after inhibition of NO synthesis. In the context of these studies, rapid tachyphylaxis is defined as the progressive loss of response elicited by second or subsequent doses of ISO. The specific aims were to determine (1) the role of endothelium-derived NOFs in the vasodilator responses elicited by systemic injections of ISO in pentobarbital-anesthetized rats, (2) whether ISO-induced vasodilation was subject to tachyphylaxis after inhibition of NO synthesis, and (3) whether administration of L-SNC would prevent tachyphylaxis to ISO after inhibition of NO synthesis.

Methods

Animals and Surgical Procedures
The protocols were approved by the University of Iowa Animal Care and Use Committee. Male Sprague-Dawley rats (Harlan, Indiana-


Experimental Protocols

All drugs were given as intravenous bolus injections. The responses elicited by each injection of ISO or L-SNC were allowed to fully recover before the next injection was given. The responses elicited by the NO synthesis inhibitor L-NAME (L-NAME) reached plateau levels by 15 to 20 minutes. As such, the first injection of ISO was given 20 minutes after the injection of L-NAME (or saline). The groups are described below on the basis of the treatments they received.

**Saline-I**

The saline-I group (n=6) received saline (0.9% NaCl IV), ISO (0.1 to 10 μg/kg IV), first dose-response [DR] curve), 8 injections of saline, and then ISO (0.1 to 10 μg/kg IV, second DR curve).

**Saline-II**

The saline-II group (n=5) received saline, ISO (0.1 to 10 μg/kg IV), 8 injections of ISO (10 μg/kg IV), and then ISO (0.1 to 10 μg/kg IV).

**L-NAME-I**

The L-NAME-I group (n=5) received L-NAME (100 μmol/kg IV), ISO (0.1 to 10 μg/kg IV), 8 injections of saline, and then ISO (0.1 to 10 μg/kg IV).

**L-NAME-II**

The L-NAME-II group (n=5) received L-NAME (100 μmol/kg IV), ISO (0.1 to 10 μg/kg IV), 8 injections of ISO (10 μg/kg IV), and then ISO (0.1 to 10 μg/kg IV).

**L-NAME-III**

The L-NAME-III group (n=5) received L-NAME (100 μmol/kg IV) and ISO (0.1 to 10 μg/kg IV). The rats then received L-SNC (1200 nmol/kg IV), which produced a fall in MAP and vascular resistance. Once the hemodynamic effects of L-SNC had subsided, the rats received ISO (10 μg/kg IV). This was repeated until rats had received 8 injections of L-SNC and ISO.

These experiments lasted for ~3 hours. Specifically, the first set of ISO injections (0.1 to 10 μg/kg IV, first DR curve) took ~30 minutes to administer. The 8 injections of saline, or ISO (10 μg/kg IV) with and without injections of L-SNC (1200 nmol/kg IV), took ~2 hours to administer. The second set of ISO injections (0.1 to 10 μg/kg IV, second DR curve) also took ~30 minutes to administer. The L-NAME–induced plateaus in MAP and vascular resistances were sustained throughout the experiments. A supplemental dose of L-NAME (50 μmol/kg IV) was given halfway through the experiments to ensure that these plateau values were maintained.

**Drugs**

All drugs were obtained from Sigma Chemical Co, except sodium pentobarbital, which was obtained from Abbott Laboratories. L-SNC was prepared as described previously.8 All drugs were dissolved and/or diluted for injection in sterile saline.

**Statistical Analyses**

The data are presented as mean±SEM. The single SEM term on each graph is prepared as described previously.7 All drugs were obtained from Sigma Chemical Co, except sodium pentobarbital, which was obtained from Abbott Laboratories. L-SNC was prepared as described previously.7 All drugs were dissolved and/or diluted for injection in sterile saline.

**Results**

**Effects of Saline or L-NAME on Resting Hemodynamic Variables**

Resting variables in the 5 groups of rats used in the present study are summarized in the Table. Resting variables were sampled until precise estimates were obtained. The values obtained before or after injection of saline or L-NAME (100 μmol/kg IV) were averaged. In subsequent phases, preinjection values for each injection of ISO or saline were determined and averaged because they were similar to one another. The initial injection of saline did not affect resting
L-NAME produced substantial increases in MAP and vascular resistances. The L-NAME–induced responses were similar in each group and remained constant thereafter. However, resting HQR values recorded during the second ISO DR curve in L-NAME–treated rats were lower than those before the injections of L-SNC because of the long-lasting effects of the last injection of L-SNC.

**Effects of L-NAME on the Hemodynamic Actions of ISO**

The responses elicited by ISO (0.1 to 10.0 µg/kg IV, first DR curve) were determined in 2 groups of saline-treated rats and in 3 groups of L-NAME (100 µmol/kg IV)–treated rats. The data from the 2 saline-treated groups of rats (n=11 rats in total) were combined as were the data from the 3 L-NAME–treated groups of rats (n=15 rats). The effects of L-NAME were similar in each group (P>0.05 for all between-group comparisons). The responses elicited by ISO were similar in the 2 saline-treated groups and in the 3 L-NAME–treated groups (P>0.05 for all between-group comparisons). Resting MAP, HQR, and MR values in saline-treated rats were 116±2 mm Hg, 108±9 mm Hg/kHz, and 66±5 mm Hg/kHz, respectively. Resting MAP, HQR, and MR values in L-NAME–treated rats were 156±3 mm Hg, 220±22 mm Hg/kHz, and 85±7 mm Hg/kHz, respectively. The values in L-NAME–treated rats were higher than the values in saline-treated rats (P<0.05 for all comparisons). Arithmetic and percent changes in MAP and resistances elicited by ISO (0.1 to 10 µg/kg IV) in saline-treated and in L-NAME–treated rats are summarized in Figure 1. ISO elicited a dose-dependent fall in MAP in both groups. The arithmetic fall in MAP elicited by ISO was similar in both groups. However, the percent fall in MAP elicited by 1.0 to 10 µg/kg doses of ISO was smaller in L-NAME–treated rats. ISO elicited a dose-dependent fall in HQR in both groups. The arithmetic fall in HQR elicited by 1.0 to 10 µg/kg doses of ISO was greater in L-NAME–treated rats, whereas the percent fall in HQR elicited by 0.5 to 10 µg/kg doses of ISO was smaller in L-NAME–treated rats. ISO produced a dose-dependent fall in MR. The arithmetic and percent falls in MR were similar in saline-treated and L-NAME–treated rats. The responses elicited by ISO (0.1 to 10 µg/kg IV) in saline-treated rats lasted for 1 minute (lower doses) to 10 minutes (higher doses). The durations of the ISO-induced responses were similar in saline-treated and in L-NAME–treated rats, except when the magnitudes of the responses were diminished in L-NAME–treated rats.

<table>
<thead>
<tr>
<th>Group, n</th>
<th>Preinjection</th>
<th>First DR Curve</th>
<th>8 Injections</th>
<th>Second DR Curve</th>
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<td>Saline-I (n=6)</td>
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<td>94±12*</td>
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</table>

Values are mean±SEM; n is the number of rats. The dose of L-NAME was 100 µmol/kg IV. Rats designated a saline–I received saline (0.9% NaCl IV), ISO (0.1–10 µg/kg IV, first DR curve), 8 injections of saline, and then ISO (0.1–10 µg/kg IV, second DR curve). Saline–II received saline, ISO (first DR curve), 8 injections of ISO (10 µg/kg IV), and ISO (second DR curve). L-NAME–I received L-NAME, ISO (first DR curve), 8 injections of saline, and then ISO (second DR curve). L-NAME–II received L-NAME, ISO (first DR curve), 8 injections of ISO (10 µg/kg IV), and then ISO (second DR curve). L-NAME–III received L-NAME, ISO (first DR curve), 8 injections of ISO (10 µg/kg IV) each preceded by an injection of L-SNC (1200 nmol/kg IV), and then ISO (second DR curve).

*P<0.05 for subsequent phases vs preinjection; †P<0.05 for second DR curve vs first DR curve.
Responses Produced by Repeated Injections of ISO in Saline-Pretreated Rats

The first injection of ISO (10 μg/kg IV) elicited a fall in MAP (−59±6%), HQR (−64±4%), and MR (−65±5%) in saline-treated rats (P<0.05 for all responses). Subsequent injections elicited similar responses (P>0.05 for all comparisons to first injection response, data not shown). The duration of the fall in MAP, HQR, and MR elicited by each injection of ISO lasted for approximately 10 minutes. The durations of the responses produced by each injection were similar to one another in these saline-treated rats (P>0.05 for all comparisons, data not shown).

Responses Produced by Repeated Injections of ISO in L-NAME-Pretreated Rats

The first injection of ISO (10 μg/kg IV) elicited a fall in MAP (−59±6%), HQR (−64±4%), and MR (−65±5%) in L-NAME–treated rats (P<0.05 for all responses). Subsequent injections elicited similar responses (P>0.05 for all comparisons to first injection response, data not shown). The falls in MAP and HQR were smaller than those in saline-treated rats (P<0.05 for all comparisons). The fall in MR was similar to that in saline-treated rats (P>0.05 for all comparisons, data not shown).

Effects of L-SNC on Tachyphylaxis to ISO in L-NAME–Pretreated Rats

The first injection of L-SNC (1200 nmol/kg IV) elicited a pronounced fall in MAP (−68±2%), HQR (−73±2%), and MR (−72±2%) in L-NAME–treated rats (P<0.05 for all responses). Each subsequent injection of L-SNC elicited similar responses (P>0.05 for all comparisons to first injection response, data not shown). The responses elicited by 8 injections of ISO (10 μg/kg IV) in L-NAME–treated rats that received L-SNC (1200 nmol/kg IV) before each injection of ISO are summarized in Figure 3. The first injection of ISO elicited a significant (P<0.05) fall in MAP (−46±4%), HQR (−59±6%), and MR (−58±7%). These responses were similar to those in L-NAME–treated rats that did not receive L-SNC (P>0.05 for all comparisons). Subsequent injections of ISO elicited similar responses in these L-SNC–treated rats.

Comparison of the First and Second ISO DR Curves in Saline-Pretreated Rats

The percent changes in MAP and vascular resistances elicited by the first and second ISO DR curves (0.1 to 10 μg/kg IV) in saline-treated rats are summarized in Figure 4. The 2 ISO DR curves were similar in rats that received 8 injections of saline between the ISO DR curves. The responses elicited by the lowest dose of ISO (second ISO DR curve) were reduced in rats that received 8 injections of ISO (10.0 μg/kg IV).
between the ISO DR curves. The responses elicited by the higher doses of ISO (second ISO DR curve) were not diminished. The responses elicited by ISO (0.1 to 10 \( \mu \text{g/kg} \) IV) lasted for \( \sim1 \) minute (lower doses) to 10 minutes (higher doses). These responses were usually of lesser duration in L-NAME–treated rats because the maximal ISO-induced responses were diminished (see below).

Comparison of the First and Second ISO DR Curves in L-NAME–Pretreated Rats

The first and second ISO DR curves (0.1 to 10 \( \mu \text{g/kg} \) IV) in L-NAME (100 \( \mu \text{mol/kg} \) IV)–treated rats that received 8 injections of saline between the 2 DR curves are summarized in the left panels of Figure 5. The 2 DR curves were identical; however, the fall in MAP elicited by 1.0 \( \mu \text{g/kg} \) (2nd ISO DR) was smaller than before the 8 injections of saline. The first and second ISO DR curves (0.1 to 10 \( \mu \text{g/kg} \) IV) in L-NAME (100 \( \mu \text{mol/kg} \) IV)–treated rats that received 8 injections of ISO (10 \( \mu \text{g/kg} \) IV) between the 2 DR curves are summarized in the right panels of Figure 5. The responses elicited by each dose of ISO (second DR curve) were markedly smaller than the values before the 8 injections of ISO. Preinjection values remained constant during these injections, and the loss of response to ISO would be evident if expressed as arithmetic changes.

Effects of Prior Injections of L-SNC on the Second ISO DR Curves in L-NAME–Pretreated Rats

The first and second ISO DR curves (0.1 to 10 \( \mu \text{g/kg} \) IV) in L-NAME (100 \( \mu \text{mol/kg} \) IV)–treated rats that received L-SNC (1200 nmol/kg IV) before each of the 8 injections of ISO (10 \( \mu \text{g/kg} \) IV) are summarized in Figure 6. The falls in MAP elicited by the 5.0 and 10.0 \( \mu \text{g/kg} \) doses of ISO (second ISO DR curve) were smaller than those before the 8 injections of ISO and L-SNC. The fall in HQR elicited by the 10 \( \mu \text{g/kg} \) dose of ISO (2nd DR curve) was smaller than that before the 8 injections of ISO and L-SNC (first DR curve). The ISO-induced changes in MR were similar before and after administration of the 8 doses of ISO and L-SNC. As such, the loss of response to ISO (second DR) was markedly less than that observed in L-NAME–treated rats that did not receive the injections of L-SNC (see Figures 3 and 4 for comparisons).

Discussion

Role of Endothelium-Derived NOFs in the Hemodynamic Actions of ISO

The present study confirms that ISO produces a dose-dependent fall in MAP and vascular resistance in pentobarbital-anesthetized rats. The percent fall in HQR produced by ISO (first DR curve) was slightly smaller in L-NAME–treated than in saline-treated rats, whereas the ISO-induced fall in MR was not. Although there is evidence that the vasodilator actions of ISO involve endothelium-derived NOFs, it appears that these factors do not play a major role in mediating the vasodilator effects of ISO in pentobarbital-anesthetized rats.
saline between the DR curves. This demonstrates that a loss of response to ISO did not occur over time. The vasodilator effects of ISO (10 μmol/kg IV) were subject to tachyphylaxis on repeated injection in L-NAME–treated rats. Moreover, the second DR curves were more shallow than the DR curves in these rats. This suggests that endothelium-derived NOFs prevent the desensitization of β-ARs or play a vital role in the resensitization of β-ARs. Long-term administration of β-AR agonists results in tachyphylaxis. This may involve the uncoupling of β-ARs from G proteins and the sequestration of β-ARs into organelles. The rapid tachyphylaxis to ISO in L-NAME–treated rats may involve the above processes.

L-SNC Prevents Tachyphylaxis to ISO in L-NAME–Pretreated Rats

L-SNC prevented tachyphylaxis to pituitary adenylate cyclase–activating polypeptide-27 in L-NAME–treated rats, whereas the NO donor, sodium nitroprusside, or the membrane-permeable cGMP analogue, 8-(4-chlorophenylthio)-cGMP, did not. The finding that L-SNC prevented tachyphylaxis to the hemodynamic actions of ISO in L-NAME–treated rats supports the contention that tachyphylaxis to ISO occurs because the vasculature is not exposed to endothelium-derived NOFs and not because of a deficiency of NO/cGMP. L-SNC alters the activity of functional proteins by nitrosation of amino acids in these proteins. Accordingly, L-SNC may prevent tachyphylaxis to ISO by nitrosation of β-ARs, cAMP-dependent protein kinase, or β-AR kinases or may facilitate the resensitization of β-ARs. The ability of S-nitrosothiols to activate stereoselective recognition sites in plasma membranes may also play a role in preventing tachyphylaxis to ISO.

Summary

The present study provides evidence that endothelium-derived NOFs prevent tachyphylaxis to ISO in vivo. Tachyphylaxis to ISO in L-NAME–treated rats may involve desensitization of β-ARs. The lack of exposure to NOFs may explain why rapid desensitization of β-ARs readily occurs in isolated cells and reconstituted receptor–G protein preparations, whereas tachyphylaxis to ISO does not readily occur on repeated injection in saline-treated rats. Hypertension and diabetes are associated with endothelial cell dysfunction and a loss of G protein–coupled receptor–G protein preparations, whereas tachyphylaxis to ISO does not readily occur on repeated injection in saline-treated rats. Tachyphylaxis to ISO in L-NAME–treated rats may involve desensitization of receptors that is due to the reduced release of endothelium-derived NOFs and neurogenically derived NOFs. The possibility that L-SNC may prevent the desensitization of β-ARs awaits appropriate molecular studies, which may confirm whether L-SNC prevents ISO-induced phosphorylation/desensitization of β-ARs.

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References


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