Rapid tachyphylaxis to hemodynamic effects of PACAP-27 after inhibition of nitric oxide synthesis

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Whalen, Erin J., Mark D. Travis, Alan Kim Johnson, and Stephen J. Lewis. Rapid tachyphylaxis to hemodynamic effects of PACAP-27 after inhibition of nitric oxide synthesis. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H2117–H2126, 1999.—The vasodilator effects of pituitary adenylate cyclase-activating polypeptide (PACAP)-27 are subject to tachyphylaxis in rats treated with the nitric oxide (NO) synthase inhibitor N\textsuperscript{-}G\textsuperscript{-}nitro-\textsuperscript{-}arginine methyl ester (L-NAME). We examined whether this tachyphylaxis could be prevented by administration of the putative endothelium-derived nitrosyl factor S-nitroso-L-cysteine (L-SNC) and whether L-SNC may exert its effects via increases in cGMP levels in vascular smooth muscle. Five doses of PACAP-27 (2 nmol/kg iv) produced pronounced vasodilator responses in saline-treated rats. These responses were not subject to tachyphylaxis. The first injection of PACAP-27 (2 nmol/kg iv) in L-NAME-treated (50 µmol/kg iv) rats produced vasodilator responses similar to those in saline-treated rats, whereas subsequent injections produced progressively smaller responses. The injection of L-SNC (1,200 nmol/kg iv) before each injection of PACAP-27 prevented tachyphylaxis in rats, whereas equihypotensive doses of the NO donor sodium nitroprusside (100 µg/kg iv) did not. The injection of the membrane-permeant cGMP analog 8-(4-chlorophenylthio)guanosine 3',5'-cyclic monophosphate (8-CPT-cGMP; 30 µmol/kg iv) to L-NAME-treated rats restored resting hemodynamic values to pre-L-NAME levels but did not prevent the development of tachyphylaxis to PACAP-27. These results suggest that nitrosyl factors prevent the development of tachyphylaxis to the hemodynamic actions of PACAP-27. These nitrosyl factors may act independently of their ability to generate cGMP in vascular smooth muscle.

**METHODS**

Rats. The protocols were approved by the University of Iowa Animal Care and Use Committee. Male Sprague-Dawley rats (Harlan, Indianapolis, IN; 250–300 g) were used in these studies.

Surgical procedures. Some rats were anesthetized with an initial injection of pentobarbital sodium (25 mg/kg ip) followed by an injection of urethan (500 mg/kg ip). These rats were supplemented with urethan (100 mg/kg iv) during the surgical procedures and the experiments as needed. Other rats were anesthetized with an injection of pentobarbital (50 mg/kg ip), and supplemental doses of pentobarbital (5 mg/kg iv) were given as needed. Polyethylene catheters were placed in the femoral vein for the administration of drugs and in the lower abdominal aorta via the femoral artery for the direct measurement of pulsatile and mean (MAP) arterial blood pressure. A midline laparotomy was performed, and pulsed Doppler flow probes were placed around the left renal artery, the superior mesenteric artery, and the lower abdominal aorta via the femoral artery for the direct measurement of pulsatile and mean (MAP) arterial blood pressure. A midline laparotomy was performed, and pulsed Doppler flow probes were placed around the left renal artery, the superior mesenteric artery, and the lower abdominal aorta via the femoral artery for the direct measurement of pulsatile and mean (MAP) arterial blood pressure. A midline laparotomy was performed, and pulsed Doppler flow probes were placed around the left renal artery, the superior mesenteric artery, and the lower abdominal aorta via the femoral artery for the direct measurement of pulsatile and mean (MAP) arterial blood pressure. A midline laparotomy was performed, and pulsed Doppler flow probes were placed around the left renal artery, the superior mesenteric artery, and the lower abdominal aorta via the femoral artery for the direct measurement of pulsatile and mean (MAP) arterial blood pressure. A midline laparotomy was performed, and pulsed Doppler flow probes were placed around the left renal artery, the superior mesenteric artery, and the lower abdominal aorta via the femoral artery for the direct measurement of pulsatile and mean (MAP) arterial blood pressure. A midline laparotomy was performed, and pulsed Doppler flow probes were placed around the left renal artery, the superior mesenteric artery, and the lower abdominal aorta via the femoral artery for the direct measurement of pulsatile and mean (MAP) arterial blood pressure.
During surgery and experimentation, animal body temperature was maintained at 37°C via a thermostat-controlled heating pad. The rats were allowed to breathe room air supplemented with 95% O2-5% CO2 via a face mask.

Experimental protocol 1. Five injections of PACAP-27 (2 nmol/kg iv) were given to urethan-anesthetized rats before and after administration of L-NAME (25 µmol/kg iv; n = 5) or L-NAME (25 µmol/kg iv; n = 7). The hemodynamic changes produced by L-NAME had reached their plateaus at this time. The injections of PACAP-27 were given ~10 min apart to allow the hypotensive and vasodilator effects of each injection of PACAP-27 to return to preinjection levels or to new plateau levels before the next injection was given.

Experimental protocol 2. One group of urethan-anesthetized rats (n = 5) received L-NAME (50 µmol/kg iv), and after 15–20 min (the time at which the hemodynamic values had reached their plateau values), a dose of L-SNC (1,200 nmol/kg iv) was given. Once the hemodynamic effects of L-SNC had subsided, the first dose of PACAP-27 (2 nmol/kg iv) was given. This procedure was repeated until five doses of L-SNC and PACAP-27 were given. The higher dose of L-NAME used in these longer protocols was given to ensure adequate blockade of NO synthesis in these longer protocols.

Experimental protocol 3. One group of urethan-anesthetized rats (n = 5) received L-NAME (50 µmol/kg iv), and after 15–20 min a dose of SNP (100 µg/kg iv) was given. Once the hemodynamic effects of SNP had subsided, the first dose of PACAP-27 (2 nmol/kg iv) was given. This procedure was repeated until five doses of SNP and PACAP-27 were given. The higher dose of L-NAME used in these studies was given to ensure adequate blockade of NO synthesis in these longer protocols.

Experimental protocol 4. Two groups of pentobarbital-anesthetized rats received L-NAME (50 µmol/kg iv), and after 15–20 min one group (n = 5) received a bolus injection of saline, and the other group (n = 5) received a bolus injection of 8-CPT-cGMP (30 µmol/kg iv). This dose of 8-CPT-cGMP caused the resting baseline parameters to return to pre-L-NAME levels (see results). After 15–20 min (at which time the hemodynamic effects of 8-CPT-cGMP had reached plateau values), these two groups received five injections of PACAP-27 (2 nmol/kg iv) given ~10 min apart. The rats that received 8-CPT-cGMP (30 µmol/kg iv) were supplemented with 8-CPT-cGMP (15 µmol/kg iv) halfway through the PACAP-27 injection protocol to keep baseline values at pre-L-NAME levels. The effects of 8-CPT-cGMP on the development of tachyphylaxis to PACAP-27 were also examined in urethan-anesthetized rats (n = 3). The protocol was identical to that described for pentobarbital-anesthetized rats.

Drugs. L-Cysteine, urethan, sodium nitrite, L-NAME, and 8-CPT-cGMP were obtained from Sigma Chemical (St. Louis, MO). Pentobarbital sodium was obtained from Abbott (Chicago, IL). PACAP-27 was obtained from Bachem (Torrance, CA). L-SNC was prepared by reacting 1 ml of 0.2 M sodium nitrite and 1 ml of 0.2 M L-cysteine (containing 100 µl of 1 N HCl), which resulted in a stable (pH ~ 3) 0.1 M stock solution (5). The stock solution was dissolved 1,000 times in saline to yield a weakly acidic injectate (pH 5–6).

Statistical analyses. The data are presented as means ± SE. The data were analyzed by repeated-measures analysis of variance followed by Student’s modified t-test with the Bonferroni correction for multiple comparisons between means (3–5).

RESULTS

Maximal hemodynamic effects produced by 5 consecutive injections of PACAP-27 in urethan-anesthetized rats before and after administration of L-NAME. The hemodynamic effects produced by five injections of PACAP-27 (2 nmol/kg iv) in urethan-anesthetized rats before and after administration of L-NAME (25 µmol/kg iv) are summarized in Fig. 1. Before administration of L-NAME, injection 1 of PACAP-27 produced pronounced falls in MAP, HQR, and MR but relatively minor falls in RR. Each subsequent injection of PACAP-27 produced similar responses (P > 0.05 for all comparisons). After administration of L-NAME, injection 1 of PACAP-27 produced hypotensive and vasodilator responses that were similar to those before administration of L-NAME (P > 0.05 for all comparisons). The vasodilator response produced by the first injection of PACAP-27 in the renal bed was greater (P < 0.05) after administration of L-NAME. However, this renal vasodilation was similar (P > 0.05) to the vasodilation produced by the fifth injection of PACAP-27 before administration of L-NAME. Each subsequent injection of PACAP-27 produced progressively and substantially smaller responses after administration of L-NAME. The hemodynamic effects of five injections of PACAP-27 (2 nmol/kg iv) were similar before and after administration of saline (P > 0.05 for all comparisons, data not shown).

Time course of hemodynamic effects produced by 5 injections of PACAP-27 before and after administration of L-NAME. The time course of the hemodynamic effects produced by the first, second, and fifth injections of PACAP-27 (2 nmol/kg iv) before and after administration of L-NAME (25 µmol/kg iv) is summarized in Fig. 3. Before administration of L-NAME, injection 1 of PACAP-27 produced decreases in MAP, HQR, and MR that lasted >4 min. Each subsequent injection of PACAP-27 produced responses of similar duration (P > 0.05 for all comparisons), except for the renal vasodilation produced by injection 5 of PACAP-27, which was of longer duration than that produced by injection 1 (P < 0.05). After administration of L-NAME, the duration of the PACAP-27-induced responses progressively diminished with each injection of PACAP-27.

Resting hemodynamic parameters before each injection of PACAP-27 and after administration of L-NAME. The resting hemodynamic parameters before each of the five injections of PACAP-27 (2 nmol/kg iv) before and after administration of L-NAME (50 µmol/kg iv) are summarized in Table 1. L-NAME increased MAP (+34 ± 6%, P < 0.05), HQR (+182 ± 17%, P < 0.05), RR (+87 ± 12%, P < 0.05), and MR (+107 ± 14%, P < 0.05). The pre-PACAP-27 injection values remained at similar levels after administration of L-NAME (P > 0.05 for all comparisons), with some exceptions. The pre-PACAP-27 injection values for injection 5 of PACAP-27 were lower than those of injection 1 (~24 ± 6%, P < 0.05). The pre-PACAP-27 injection values for injection 4 were higher than those of injection 1 (+28 ± 7%, P < 0.05) because the baseline MR returned to higher values after recovery from the vasodilator effects of injection 3. The pre-PACAP-27 injection values for the fifth injection of PACAP-27 were higher than the pre-PACAP-27 injection values for the fourth injection of PACAP-27 (+14 ± 3%, P < 0.05).

Effects of L-SNC on PACAP-27-induced tachyphylaxis in L-NAME-treated, urethan-anesthetized rats. Urethan-anesthetized rats received L-NAME (50 µmol/kg iv) and
then five injections of PACAP-27 (2 nmol/kg iv), each preceded by an injection of L-SNC (1,200 nmol/kg iv). The hemodynamic effects of L-SNC or PACAP-27 were allowed to return to preinjection levels or to a new resting plateau (see next section) before the next injection was given. The maximal effects produced by the five injections of L-SNC are shown in the left panels of Fig. 3, and the hemodynamic effects produced by the five injections of PACAP-27 (2 nmol/kg iv) are shown in the right panels. The first injection of L-SNC produced pronounced falls in MAP and vascular resistances (P < 0.05 for all comparisons). Each subsequent injection of L-SNC produced similar responses (P > 0.05 for all between-injection comparisons). The first injection of PACAP-27 produced pronounced falls in MAP and vascular resistances (P < 0.05 for all comparisons). Each subsequent injection of PACAP-27 produced similar responses (P > 0.05 for all comparisons). The maximal effects produced by the subsequent injections of PACAP-27 were not subject to tachyphylaxis in the L-NAME-treated rats that received L-SNC before each injection of PACAP-27 (P > 0.05 for all between-injection comparisons).

Resting parameters in L-NAME-treated rats that received L-SNC before each of 5 injections of PACAP-27 are summarized in Table 2. L-NAME produced a substantial increase in MAP (+44 ± 5% of 1st pre-L-SNC injection value, P < 0.05), HQR (+136 ± 18%, P < 0.05), RR (+141 ± 15%, P < 0.05) and MR (+236 ± 28%, P < 0.05). The pre-L-SNC and pre-PACAP-27 injection values remained at similar levels in these L-NAME-treated rats (P > 0.05 for all comparisons), with one exception. The preinjection HQR values for the fifth injection of L-SNC and PACAP-27 were lower than the preinjection values for the first injection of L-SNC (-17 ± 5%, P < 0.05) and PACAP-27 (-16 ± 4%, P < 0.05). This is because the resting HQR returned to a lower resting value after recovery from the vasodilator effects of the fourth injection of PACAP-27.

Fig. 1. Maximal changes (Δ) in mean arterial pressure (MAP) and hindquarter (HQR), renal (RR), and mesenteric (MR) vascular resistances produced by 5 successive injections of pituitary adenylate cyclase-activating polypeptide (PACAP)-27 (2 nmol/kg iv) in urethan-anesthetized rats (n = 5) before (A) and after (B) injection of aN₂-nitro-L-arginine methyl ester (L-NAME; 25 µmol/kg iv). Data are expressed as means ± SE of percent changes from preinjection values. *P < 0.05, 2nd-5th injections vs. 1st injection of PACAP-27. †P < 0.05, 1st injection post-L-NAME vs. 1st injection pre-L-NAME.
Table 1. Resting hemodynamic values before each injection of PACAP-27 before and after administration of L-NAME

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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<td>MAP, mmHg</td>
<td>Pre-L-NAME</td>
<td>101 ± 4</td>
<td>103 ± 3</td>
<td>105 ± 4</td>
<td>102 ± 3</td>
<td>103 ± 4</td>
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<td>136 ± 3*</td>
<td>139 ± 4*</td>
<td>140 ± 5*</td>
<td>137 ± 3*</td>
</tr>
<tr>
<td>HQR, mmHg/kHz</td>
<td>Pre-L-NAME</td>
<td>34 ± 5</td>
<td>36 ± 4</td>
<td>35 ± 3</td>
<td>30 ± 3</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>RR, mmHg/kHz</td>
<td>Post-L-NAME</td>
<td>79 ± 6*</td>
<td>82 ± 9*</td>
<td>78 ± 7*</td>
<td>69 ± 6*</td>
<td>63 ± 5†</td>
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<tr>
<td>MR, mmHg/kHz</td>
<td>Pre-L-NAME</td>
<td>60 ± 8</td>
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<td>61 ± 10</td>
<td>59 ± 7</td>
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<td></td>
<td>Post-L-NAME</td>
<td>108 ± 10*</td>
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<td>Post-L-NAME</td>
<td>73 ± 7*</td>
<td>79 ± 8*</td>
<td>83 ± 6*</td>
<td>96 ± 5†</td>
<td>110 ± 7†</td>
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</table>

Data represent means ± SE of resting mean arterial blood pressure (MAP) and hindquarter (HQR), renal (RR) and mesenteric (MR) resistance values before each of 5 injections of pituitary adenylate cyclase-activating polypeptide (PACAP-27; 2 nmol/kg iv) before and after injection of N⁷-nitro-L-arginine methyl ester (L-NAME; 25 µmol/kg iv) in urethan-anesthetized rats (n = 7). *P < 0.05, post-L-NAME values vs. pre-L-NAME values; †P < 0.05, subsequent PACAP-27 preinjection values vs. 1st PACAP-27 preinjection value.
Effects of SNP on PACAP-27-induced tachyphylaxis in L-NAME-treated, urethan-anesthetized rats. A group of urethan-anesthetized rats received L-NAME (50 µmol/kg iv) and then five injections of PACAP-27 (2 nmol/kg iv), each preceded by an injection of SNP (100 µg/kg iv). The maximal hemodynamic effects produced by the five injections of SNP are shown in the left panels of Fig. 4, and the effects of the five injections of PACAP-27 (2 nmol/kg iv) are shown in the right panels of this figure. The first injection of SNP produced pronounced falls in MAP and resistances in each bed (P < 0.05 for all comparisons). These responses were similar to or greater in magnitude than those produced by L-SNC (see Fig. 3). Each subsequent injection of SNP produced similar responses (P > 0.05 for all between-injection comparisons). The first injection of PACAP-27 produced pronounced falls in MAP and vascular resistances (P < 0.05 for all comparisons) that were similar to those in rats that received L-NAME only (P > 0.05 for all comparisons). However, SNP did not prevent the development of tachyphylaxis to the hemodynamic effects of subsequent doses of PACAP-27. L-NAME produced increases in resting MAP and vascular resistances similar to those described above (P > 0.05 for all comparisons). 

Table 2. Resting hemodynamic values before each injection of L-SNC and PACAP-27 in L-NAME-treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Injection No.</th>
<th>Parameter</th>
<th>MAP, mmHg</th>
<th>HQR, mmHg/kHz</th>
<th>RR, mmHg/kHz</th>
<th>MR, mmHg/kHz</th>
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<td>Pre-L-NAME</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Post-L-NAME</td>
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<td>Pre-SNC</td>
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<td>106 ± 7*</td>
<td>74 ± 6*</td>
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<td>2</td>
<td>143 ± 5*</td>
<td>92 ± 7*</td>
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<td>79 ± 10*</td>
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<td>3</td>
<td>141 ± 4*</td>
<td>90 ± 8*</td>
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<td>81 ± 9*</td>
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<td></td>
<td>4</td>
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<td>5</td>
<td>138 ± 5*</td>
<td>74 ± 7*</td>
<td>110 ± 9*</td>
<td>80 ± 8*</td>
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</tr>
<tr>
<td>Pre-PACAP-27</td>
<td>1</td>
<td>140 ± 5*</td>
<td>89 ± 9*</td>
<td>112 ± 10*</td>
<td>72 ± 8*</td>
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<td>143 ± 4*</td>
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Data represent means ± SE of resting MAP, HQR, RR, and MR values before each injection of L-SNC; 1200 nmol/kg iv) and PACAP-27 (2 nmol/kg iv) in L-NAME-treated (50 µmol/kg iv), urethan-anesthetized rats (n = 5). *P < 0.05, post-L-NAME vs. pre-L-NAME; †P < 0.05, subsequent L-SNC or PACAP-27 preinjection values vs. 1st preinjection values.
comparisons). The pre-SNP and pre-PACAP-27 injection values remained constant in these l-NAME-treated rats (P < 0.05 for all comparisons). More specifically, the resting hemodynamic values returned to preinjection values after recovery from the hemodynamic effects of these compounds (P > 0.05 for all comparisons).

Maximal vasodilator responses produced by 5 injections of PACAP-27 in l-NAME-treated rats that received saline or 8-CPT-cGMP before injection of PACAP-27. The maximal changes in HQR produced by five consecutive injections of PACAP-27 (2 nmol/kg iv) in two groups of pentobarbital-anesthetized rats before and after administration of l-NAME (50 µmol/kg iv) are summarized in Fig. 5. One group of l-NAME-treated rats received a bolus injection of saline before administration of the five injections of PACAP-27. The other group of l-NAME-treated rats (n = 5) received a bolus injection of 8-CPT-cGMP (30 µmol/kg iv) before administration of the five injections of PACAP-27. This dose of 8-CPT-cGMP lowered MAP and HQR to pre-l-NAME values (see next section). The first injection of PACAP-27 produced pronounced falls in HQR and MAP in the l-NAME-treated rats that received an injection of saline before the first injection of PACAP-27 (P < 0.05 for both responses). As can be seen, tachyphylaxis readily occurred to the hemodynamic effects of PACAP-27 on repeated administration of this peptide. The maximal falls in HQR produced by the first injection of PACAP-27 in l-NAME-treated rats that received 8-CPT-cGMP were similar to those in rats that received saline before the first injection of the peptide (P > 0.05 for all comparisons). As can be seen, 8-CPT-cGMP did not prevent the development of tachyphylaxis to the hindquarter vasodilator effects of PACAP-27 in l-NAME-treated rats. More specifically, the vasodilator responses produced by each injection of PACAP-27 were similar in saline- and 8-CPT-cGMP-treated rats (P > 0.05 for all comparisons).

The maximal changes in MR produced by five consecutive injections of PACAP-27 (2 nmol/kg iv) in the above-described groups of rats are summarized in Fig. 6. This dose of 8-CPT-cGMP lowered MAP and MR to pre-l-NAME values (see next section). The first injection of PACAP-27 produced pronounced falls in MR and MAP in the l-NAME-treated rats that received an
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injection of saline before the first injection of PACAP-27 (P < 0.05 for both responses). Tachyphylaxis readily occurred to the vasodilator effects of PACAP-27 on repeated administration of this peptide. The maximal falls in MR produced by the first injection of PACAP-27 in L-NAME-treated rats that received 8-CPT-cGMP were similar to those in rats that received saline (P > 0.05). 8-CPT-cGMP did not prevent the development of tachyphylaxis to the vasodilator effects of PACAP-27 in the mesenteric bed of these L-NAME-treated rats. More specifically, the vasodilator responses produced by each injection of PACAP-27 were similar in saline- and 8-CPT-cGMP-treated rats (P > 0.05 for all comparisons). The pattern of responses obtained in urethane-anesthetized rats (n = 3) were very similar to those described in these pentobarbital-anesthetized rats.

Resting hemodynamic values before each of 5 consecutive injections of PACAP-27 in L-NAME-treated rats that received bolus injection of saline or 8-CPT-cGMP before injection of PACAP-27. The resting hemodynamic parameters before each of five injections of PACAP-27 (2 nmol/kg iv) in pentobarbital-anesthetized, L-NAME-treated (50 µmol/kg iv) rats that received an injection of saline before PACAP-27 are summarized in Table 3. L-NAME increased MAP (+39 ± 6%, P < 0.05), HQR (+131 ± 23%, P < 0.05), and MR (+177 ± 19%, P < 0.05). The pre-PACAP-27 MAP values were similar in these L-NAME-treated rats (P > 0.05 for all comparisons). More specifically, resting MAP returned to preinjection values after recovery from the hypotensive effects of each injection of PACAP-27. The preinjection HQR values for injection 4 of PACAP-27 were lower than those of injection 1 (−16 ± 4%, P < 0.05). This is because the resting HQR returned to a lower resting value after recovery from the vasodilator effects of injection 3 of PACAP-27. The preinjection HQR values for injection 5 of PACAP-27 were lower than those of injection 1 (−19 ± 4%, P < 0.05). This is because the resting HQR remained at lower resting values after recovery from the vasodilator effects of injection 4. The preinjection MR values for injection 4 of PACAP-27 were higher than the preinjection values of injection 1 (+32 ± 8%, P < 0.05). This is because the resting MR returned to a higher resting value after recovery from the vasodilator effects of injection 3. The preinjection MR values for injection 5 of PACAP-27 were also higher than the preinjection values of injection 1 (+45 ± 9%, P < 0.05). This is because the resting MR remained at higher resting values after recovery from the vasodilator effects of injection 4 of PACAP-27.

The resting hemodynamic parameters before each of the five injections of PACAP-27 (2 nmol/kg iv) in pentobarbital-anesthetized, L-NAME-treated (50 µmol/kg iv) rats that received an injection of 8-CPT-cGMP (30 µmol/kg iv) before the injection of PACAP-27 are also summarized in Table 3. L-NAME increased MAP (+38 ± 3%, P < 0.05), HQR (+91 ± 11%, P < 0.05), and MR (+228 ± 26%, P < 0.05). 8-CPT-cGMP produced prompt reductions in MAP (−35 ± 7%, P < 0.05), HQR (−41 ± 6%, P < 0.05), and MR (−58 ± 9%, P < 0.05). Resting MAP and resistance values after the injection of 8-CPT-cGMP were similar to those of L-NAME values (P > 0.05 for all comparisons). The
MAP and HQR values did not change over the time in which the five injections of PACAP-27 were given (P > 0.05 for all comparisons). However, the preinjection MR values for injections 4 and 5 of PACAP-27 were higher than those before injection 1 (+56 ± 18% and +98 ± 27%, respectively; P < 0.05). This steady increase in baseline MR occurred even though a 15 µmol/kg dose of 8-CPT-cGMP was given before the third injection of PACAP-27 was administered.

**DISCUSSION**

The hypotensive and vasodilator effects of PACAP-27 (2 nmol/kg iv) were not subject to tachyphylaxis in saline-treated, urethan-anesthetized rats. The hemodynamic effects of the first injection of PACAP-27 in L-NAME-treated rats were similar to those in saline-treated rats. This supports evidence that the vasodilator effects of PACAP-27 are mediated by Gs protein-coupled receptors rather than by endothelium-derived relaxing factors (13, 14, 19, 29). Tachyphylaxis rapidly developed to the hemodynamic effects of PACAP-27 in L-NAME-treated rats. In addition, the injection of L-SNC before each injection of PACAP-27 prevented tachyphylaxis to PACAP-27. This suggests that tachyphylaxis to PACAP-27 in L-NAME-treated rats was caused by the loss of exposure of VSM to endothelium-derived nitrosyl factors. A high dose of L-SNC was given to generate as much cGMP in VSM as possible. In addition, the pronounced hemodynamic effects of each injection of L-SNC were allowed to subside before the subsequent injection of PACAP-27 was given. This was done to determine whether the mechanisms by which PACAP-27 reduced MAP and HQR values did not change over the time in which the five injections of PACAP-27 were given (P > 0.05 for all comparisons).

**Table 3.** Resting parameters in L-NAME-treated rats that received saline or 8-CPT-cGMP before receiving 5 injections of PACAP-27

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
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<th>Post-NAME</th>
<th>Pre-PACAP-27 Injection Values</th>
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<tr>
<td>Saline</td>
<td>MAP, mmHg</td>
<td>112 ± 4</td>
<td>156 ± 6*</td>
<td>154 ± 5*</td>
</tr>
<tr>
<td></td>
<td>HQR, mmHg/kHz</td>
<td>98 ± 9</td>
<td>226 ± 15*</td>
<td>210 ± 14*</td>
</tr>
<tr>
<td></td>
<td>MR, mmHg/kHz</td>
<td>30 ± 4</td>
<td>83 ± 6*</td>
<td>86 ± 5*</td>
</tr>
<tr>
<td>8-CPT-cGMP</td>
<td>MAP, mmHg</td>
<td>109 ± 7</td>
<td>148 ± 5*</td>
<td>96 ± 6</td>
</tr>
<tr>
<td></td>
<td>HQR, mmHg/kHz</td>
<td>77 ± 9</td>
<td>147 ± 20*</td>
<td>88 ± 9</td>
</tr>
<tr>
<td></td>
<td>MR, mmHg/kHz</td>
<td>20 ± 2</td>
<td>66 ± 8*</td>
<td>25 ± 3</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE of resting MAP, HQR, and MR values before each of 5 injections of PACAP-27 in L-NAME-treated rats (n = 5) that received bolus injection of saline (0.9% NaCl iv) before 1st injection of PACAP-27 and in L-NAME-treated rats (n = 5) that received bolus injection of 8-CPT-cGMP (30 µmol/kg iv) before 1st injection of PACAP-27. Note that 1st post-PACAP-27 injection value represents resting value after injection of saline or 8-CPT-cGMP. *P < 0.05, pre-PACAP-27 injection value vs. post-NAME values; †P < 0.05, subsequent pre-PACAP-27 injection values vs. 1st pre-PACAP-27 injection value.
L-SNC relaxes VSM (5, 9) could be separated from those that prevent tachyphylaxis to PACAP-27. The vasorelaxant effects of S-nitrosothiols in vitro involve their decomposition to NO, which relaxes VSM by the generation of cGMP (9, 26). In theory, nitrosyl factors may prevent tachyphylaxis to PACAP-27 by activation of a cGMP-dependent protein kinase (6). The finding that L-SNC prevented tachyphylaxis to PACAP-27 even though the hemodynamic effects of the S-nitrosothiol had subsided suggests that this process was cGMP independent. The changes in intracellular cGMP levels should parallel the L-SNC-mediated falls in vascular resistances. Accordingly, cGMP levels should peak at the time of maximal vasodilation and should return to preinjection values after recovery from the vasodilator effects of L-SNC.

The injection of a large dose of the NO donor SNP before each injection of PACAP-27 did not prevent tachyphylaxis to the Gs protein-coupled receptor agonist. Each dose of SNP produced pronounced hemodynamic responses (equal to or greater than those produced by L-SNC) that were presumably caused by the generation of cGMP in VSM (26). It should be noted that SNP is not simply an NO donor (26), and the results with this compound should be taken with some caution. However, it was also evident that the administration of the membrane-permeant cGMP analog 8-CPT-cGMP was also unable to prevent the development of tachyphylaxis to PACAP-27 in the L-NAME-treated rats. These results suggest that L-SNC prevented tachyphylaxis to PACAP-27 by cGMP-independent mechanisms. The 8-CPT-cGMP experiments also provided evidence that the L-NAME-induced increases in vascular resistances were not responsible for the development of tachyphylaxis to PACAP-27. More specifically, resting HQR remained at pre-L-NAME levels during the injections of PACAP-27 in these 8-CPT-cGMP-treated rats. Tachyphylaxis to the vasodilator effects of PACAP-27 occurred in these rats even though baseline HQR was at pre-L-NAME levels. The preinjection MR levels for the first three injections of PACAP-27 were similar to pre-L-NAME levels. This is important because near-maximal tachyphylaxis was obtained with the third injection of PACAP-27. However, the resting preinjection MR values for the fourth and fifth injections of PACAP-27 were higher than pre-L-NAME levels. This rise in baseline MR may not have been caused by the loss of potency of 8-CPT-cGMP, because another injection of 8-CPT-cGMP given about halfway through the PACAP-27 injection protocol did not prevent the increase in baseline MR. The increase in baseline MR may have been caused by some active vasoconstrictor process.

Although tachyphylaxis to PACAP-27 was not observed in saline-treated rats, this does not preclude the possibility that PACAP receptors were desensitized. The rapid desensitization of Gs protein-coupled β-adrenoceptors in membrane preparations is caused by the phosphorylation of the receptors by cAMP-dependent protein kinase (PKA) and by G protein-coupled receptor kinases (GRK; Refs. 8, 20, 22). The phosphorylated receptor cannot couple to Gs proteins (8, 20, 22). The desensitized receptor is sequestered and dephosphorylated before reincorporation into the plasma membrane (8). At present, there is no evidence that PACAP receptors are phosphorylated by PKA or GRK, although these receptors contain amino acids that are subject to phosphorylation in other receptors (24). The lack of tachyphylaxis to PACAP-27 in saline-treated rats may be because 1) PACAP-27 does not desensitize PACAP receptors under normal conditions; 2) five injections of PACAP-27 (2 nmol/kg iv) do not desensitize enough receptors for a loss of response to be evident; or 3) PACAP receptors desensitized by each injection of PACAP-27 are rapidly dephosphorylated and reincorporated into the plasma membranes so that the next injection of PACAP-27 has a full complement of receptors to activate.

The processes responsible for tachyphylaxis to PACAP-27 in L-NAME-treated rats and the mechanisms by which nitrosyl factors prevent this tachyphylaxis remain to be determined. We have obtained preliminary evidence that tachyphylaxis to PACAP-27 is not caused by the loss of biological activity of cAMP generated within VSM (30). It is possible that tachyphylaxis to PACAP-27 is caused by the failure of the agonist-occupied receptor to generate cAMP. This may be caused by 1) PKA- or GRK-mediated phosphorylation of PACAP receptors that prevents the receptor from coupling to Gs proteins, 2) inability of Gs proteins to activate adenylate cyclase, or 3) inhibition of intracellular processes that dephosphorylate and reincorporate the receptor into the plasma membrane (8, 20, 22). It is possible that endothelium-derived and neurogenically derived nitrosyl factors (3, 4, 21) prevent tachyphylaxis to PACAP-27 by the activation of stereoselective recognition sites in the vasculature (5, 26, 27) or by nitrosation of amino acids in the receptor or other elements in the signal transduction cascade (10, 12, 15, 25). Although it is possible that NO synthase directly synthesizes S-nitrosothiols and that NO may be oxidized to a nitrosating agent (i.e., NO; ; Refs. 9, 18, 25), there is no conclusive evidence that this occurs under physiological conditions. Moreover, there is no evidence that the PACAP receptor is nitrosated. However, this receptor does contain cysteine residues that, in theory, would be susceptible to nitrosation (24). The nitrosation of serine, threonine, and tyrosine residues in Gs protein-coupled receptors may prevent their phosphorylation. In addition, the nitrosation of cysteine residues may prevent PKA or GRK from coupling to the receptor and phosphorylating amino acids. The possible mechanisms by which S-nitrosothiols prevent tachyphylaxis to PACAP-27 include the following: 1) nitrosation of the PACAP receptor may prevent PKA or GRK from phosphorylating the receptor; 2) nitrosation of Gs proteins or adenylate cyclase may be important in the coupling of these proteins; and 3) nitrosation events regulate the phosphatase activity or the processes that reincorporate the receptor into plasma membranes.

In summary, this study provides evidence that tachyphylaxis to PACAP-27 in L-NAME-treated rats may be caused by the loss of influence of endothelium-derived nitrosyl factors on the processes that desensitize PACAP
receptors. We have obtained preliminary evidence that the vasodilator actions of isoproterenol are subject to tachyphylaxis in l-NAME-treated rats and that l-SNC prevents this tachyphylaxis from occurring (31, 32). It therefore appears that nitrosyl factors may prevent the desensitization of a variety of Gs protein-coupled receptors in vivo. Disease states such as hypertension (11) are associated with compromised endothelial cell function and a loss of Gs protein-coupled receptor-mediated vasodilation. Our findings suggest that the loss of vasodilator potency of Gs protein-coupled receptor agonists in disease states may be caused by desensitization of these receptors as a consequence of reduced release of endothelium-derived nitrosyl factors.

The authors acknowledge the expert assistance of Mike Burdham in the preparation of the figures.

This work was supported in part by grants from the National Institutes of Health (HL-14388, HL-57472, and DK-54759), from the National Aeronautics and Space Administration (NAG5–6171), and from the Office of Naval Research (N00014–97–1-0145).

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Received 18 May 1998; accepted in final form 16 February 1999.

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