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Effects of nitric oxide synthase inhibition on sympathetically-mediated tachycardia

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Abstract

The aim of the present study was to determine whether inhibition of nitric oxide (NO) synthesis directly alters the tachycardia produced by sympathetically-derived norepinephrine. The NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; 50 μmol/kg, iv), produced a marked rise in mean arterial blood pressure. This pressor response was associated with a fall in heart rate which involved the withdrawal of cardiac sympathetic nerve activity. The NO-donor, sodium nitroprusside (5 μg/kg, iv), produced a pronounced fall in mean arterial blood pressure but only a minor increase in heart rate. The β-adrenoceptor agonist, isoproterenol (0.5 μmol/kg, iv), and the membrane-permeable cAMP analogue, 8-(4-chlorophenylthiol)-cAMP (10 μmol/kg, iv), produced falls in mean arterial blood pressure and pronounced increases in heart rate. The indirectly acting sympathomimetic agent, tyramine (0.5 mg/kg, iv), produced a pressor response and a tachycardia. The effects of sodium nitroprusside, tyramine, isoproterenol and 8-(4-chlorophenylthiol)-cAMP on mean arterial blood pressure were not markedly affected by L-NAME. However, the tachycardia produced by these agents was considerably exaggerated in the presence of this NO synthesis inhibitor. These findings suggest that L-NAME potentiates the tachycardia produced by sympathetically-derived norepinephrine. The increased responsiveness to norepinephrine may involve (i) a rapid up-regulation of cardiac β_1-adrenoceptors and cAMP signaling in cardiac pacemaker cells due to the loss of the inhibitory influence of cardiac NO, and (ii) the up-regulation of β_1-adrenoceptor-mediated signal transduction processes in response to the L-NAME-induced withdrawal of cardiac sympathetic nerve activity.

Keywords: β_1-Adrenoceptor; Heart rate; Nitric oxide (NO); cAMP; cGMP; (In vivo)

1. Introduction

Norepinephrine released from cardiac sympathetic nerves increases heart rate mainly by activation of cardiac G_s protein-coupled β_1-adrenoceptors which increase adenylate cyclase activity (Harden, 1983). β-Adrenoceptor agonists increase pacemaker rate by alteration of ion-channel activity including the cAMP-mediated activation of L-type voltage-sensitive Ca^{2+}-channels (Giles and Shibata, 1981; DiFransesco, 1993; Hartzell, 1993; Irisawa et al., 1993). The tachycardia produced by the systemic injection of the membrane permeable cAMP analogue, 8-(4-chlorophenylthiol)-cAMP, is augmented after blockade of cardiac β-adrenoceptors which suggests that the activation of β-adrenoceptors regulates the potency of cAMP within the heart (Whalen et al., 1998).

The increase in intracellular Ca^{2+} resulting from the β-adrenoceptor-mediated activation of L-type voltage-sensitive Ca^{2+}-channels would stimulate the activity of nitric oxide (NO) synthase (Moncada et al., 1991) in cardiac pacemaker cells and myocytes (Schulz et al., 1992; Balligand et al., 1993, 1995; Han et al., 1994). NO generated within isolated cardiac pacemaker cells and myocytes markedly diminishes the chronotropic and inotropic effects of β-adrenoceptor agonists and these effects appear to be mediated by increases in cGMP (Balligand et al., 1993, 1995; Brady et al., 1993; Grocott-Mason et al., 1994; Han et al., 1994; Hare et al., 1995). Moreover, intracellular cGMP counteracts the effects of cAMP in the heart (Hare et al., 1995). Taken together, these results suggest that blockade of β-adrenoceptors may indirectly diminish NO synthesis by reducing Ca^{2+} influx into cardiac pacemaker

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cells and that the resultant loss of cGMP would enhance cAMP signaling.

The tachycardia produced by centrally-mediated activation of cardiac sympathetic drive is augmented in rats treated with the NO synthesis inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) (Davisson et al., 1994). However, it is not known whether this is due to an increase in norepinephrine release from sympathetic nerve terminals or to an exaggerated responsiveness of the heart to this β-adrenoceptor agonist. The aim of this study was to determine the mechanisms by which L-NAME facilitates the tachycardia produced by sympathetically-derived norepinephrine. We determined the changes in heart rate and mean arterial blood pressure produced by systemic injections of (i) the NO-donor, sodium nitroprusside, (ii) the indirectly acting sympathomimetic agent, tyramine (Hayes et al., 1984), (iii) the β-adrenoceptor agonist, isoproterenol (Harden, 1983), and (iv) 8-(4-chlorophenylthiol)-cAMP (Whalen et al., 1998) in pentobarbital-anesthetized rats treated with saline or L-NAME (50 μmol/kg, iv). We have provided evidence that the tachycardia produced by isoproterenol and 8-(4-chlorophenylthiol)-cAMP in pentobarbital-anesthetized rats is due mainly to the direct effects of these compounds on the heart rather than by direct or baroreceptor reflex-mediated changes in autonomic nerve activity (Whalen et al., 1998).

2. Materials and methods

2.1. Rats and surgical procedures

The protocols described below were approved by the University of Iowa Animal Care and Use Committee. Male Sprague–Dawley rats (250–350 g; $n = 57$) were anesthetized with pentobarbital (50 mg/kg, i.p.) and polyethylene catheters were placed in the femoral vein to administer drugs and in the lower abdominal aorta via the femoral artery to measure pulsatile and mean arterial pressure and the determination of heart rate. The body temperature of the rats was maintained at 37°C by a heating pad and carboxygen (95% O_2–5% CO_2) was administered via a face mask. The arterial catheter was connected to a Beckman Dynograph-coupled pressure transducer (Cobe Laboratories) for the measurement of pulsatile and mean arterial blood pressure. Heart rate was determined from the pulsatile arterial pressure by a cardiotachometer.

2.2. Experimental protocols

2.2.1. General comments

Since the aim of this study was to determine whether L-NAME would augment the tachycardia produced by sodium nitroprusside, isoproterenol, 8-(4-chlorophenylthiol)-cAMP and tyramine, the doses of these agents was based upon the results of preliminary studies which demonstrated that they produced consistent but submaximal increases in heart rate. The cardiovascular effects of L-NAME, methyl-atropine and propranolol were allowed to reach their plateau levels before the test agents were given.

2.2.2. Protocol 1

The effects of L-NAME (50 μmol/kg, iv) on mean arterial blood pressure and heart rate were determined in rats treated with saline (0.9% NaCl, w/v; $n = 6$), the muscarinic acetylcholine receptor antagonist, methyl-atropine (1 mg/kg, iv; $n = 6$), or the β-adrenoceptor antagonist, propranolol (1 mg/kg, iv; $n = 7$).

2.2.3. Protocol 2

The cardiovascular effects of sodium nitroprusside (5 μg/kg, iv) were determined in saline ($n = 7$) or L-NAME-treated (50 μmol/kg, iv; $n = 7$) rats before and after administration of the β-adrenoceptor antagonist, propranolol (1 mg/kg, iv).

2.2.4. Protocol 3

The cardiovascular effects of isoproterenol (0.5 μg/kg, iv) were determined in rats pretreated with saline ($n = 5$) or L-NAME (50 μmol/kg, iv; $n = 5$).

2.2.5. Protocol 4

The cardiovascular effects of 8-(4-chlorophenylthiol)-cAMP (10 μmol/kg, iv) were determined in rats treated with saline ($n = 5$) or L-NAME (50 μmol/kg, iv; $n = 5$).

2.2.6. Protocol 5

The cardiovascular effects of tyramine (50 μg/kg, iv) were determined in rats treated with saline ($n = 5$), propranolol (1 mg/kg, iv; $n = 6$) or L-NAME (50 μmol/kg, iv; $n = 7$).

2.3. Drugs

Sterile saline, sodium pentobarbital and sodium nitroprusside were obtained from Abbott Laboratories (North Chicago, IL). Isoproterenol, methyl-atropine, propranolol, tyramine, L-NAME and 8-(4-chlorophenylthiol)-cAMP were obtained from Sigma (St. Louis, MO).

2.4. Statistical analyses

The data are presented as the mean ± S.E.M. 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 (Kooy and Lewis, 1996).

3. Results

3.1. Effects of L-NAME on resting mean arterial blood pressure and heart rate in rats pretreated with saline, methyl-atropine or propranolol

The effects of L-NAME (50 μmol/kg, iv) on mean arterial blood pressure and heart rate of rats pretreated with saline (0.9% NaCl, w/v, iv), methyl-atropine (1 mg/kg, iv) or propranolol (1 mg/kg, iv) are summarized in Table 1. The administration of saline did not affect mean arterial blood pressure or heart rate ($P > 0.05$ for both comparisons). The subsequent administration of L-NAME to these saline-treated rats produced an increase in mean arterial blood pressure which was associated with a fall in heart rate ($P < 0.05$ for both responses). Methyl-atropine did not affect mean arterial blood pressure ($P > 0.05$), but produced an increase in heart rate ($P < 0.05$). The subsequent administration of L-NAME produced an increase in mean arterial blood pressure and a decrease in heart rate ($P < 0.05$ for both responses) which were of similar magnitude to those in saline-treated rats ($P > 0.05$ for both comparisons). Propranolol did not affect mean arterial blood pressure ($P > 0.05$) but produced a decrease in heart rate ($P < 0.05$). The subsequent administration of L-NAME produced an increase in mean arterial blood pressure and a decrease in heart rate ($P < 0.05$ for both responses). The hypertension was similar to that observed in saline-treated rats ($P > 0.05$ for both comparisons). However, the L-NAME-induced bradycardia was smaller than in saline-treated rats ($P < 0.05$).

3.2. The effects of sodium nitroprusside on mean arterial blood pressure and heart rate of rats pretreated with L-NAME and propranolol

The administration of saline did not affect resting cardiovascular parameters. The resting mean arterial blood pressure values of rats ($n = 7$) before and after administration of saline were 105 ± 3 and 104 ± 3 mm Hg, respectively ($-1 \pm 2\%$, $P > 0.05$). The resting heart rate values before and after administration of saline were 346 ± 12 and 343 ± 10 bpm, respectively ($0 \pm 3\%$, $P > 0.05$). The subsequent administration of propranolol did not affect resting mean arterial blood pressure (102 ± 3 mm Hg, $-1 \pm 2\%$ of post-saline values, $P < 0.05$), but lowered resting heart rate (300 ± 12 bpm, $-14 \pm 3\%$ of post-saline values, $P < 0.05$).

L-NAME (50 μmol/kg, iv) produced a pronounced and sustained increase in mean arterial blood pressure which was accompanied by a bradycardia. The resting mean arterial blood pressure values of rats ($n = 7$) before and after administration of L-NAME were 103 ± 3 and 140 ± 5 mm Hg, respectively ($+39 \pm 8\%$, $P < 0.05$). The resting heart rate values before and after administration of L-NAME were 377 ± 14 and 333 ± 10 bpm, respectively ($-12 \pm 2\%$, $P < 0.05$). The subsequent administration of propranolol did not affect resting mean arterial blood pressure (135 ± 3 mm Hg, $-1 \pm 2\%$ of post-L-NAME values, $P < 0.05$), but lowered resting heart rate slightly (317 ± 12 bpm, $-5 \pm 1\%$ of post-L-NAME values, $P < 0.05$).

The effects of sodium nitroprusside (5 g/kg, iv) on mean arterial blood pressure and heart rate before and after the administration of saline or L-NAME (50 μmol/kg, iv) are summarized in Table 2. Prior to the administration of saline or L-NAME, sodium nitroprusside produced pronounced falls in mean arterial blood pressure which were accompanied by minor increases in heart rate. The sodium nitroprusside-induced depressor responses were similar both before and after administration of saline ($P < 0.05$ for all comparisons) and before and after treatment with L-NAME ($P > 0.05$). However, the sodium nitroprusside-induced falls in mean arterial blood pressure were now accompanied by greater increases in heart rate ($P < 0.05$

Table 1
Effects of L-NAME on mean arterial blood pressure and heart rate in rats pretreated with saline, methyl-atropine or propranolol

Pretreatment	Parameter	Pre-L-NAME			Post-L-NAME	
		Pre	Post	%Change	Post	%Change
Saline	MAP (mm Hg)	120 ± 3	118 ± 4	-2 ± 3	148 ± 5	$+25 \pm 6^a$
	HR (bpm)	336 ± 10	334 ± 12	-1 ± 2	292 ± 11	-13 ± 2^a
Methyl-atropine	MAP (mm Hg)	122 ± 4	120 ± 5	-2 ± 3	138 ± 4	$+15 \pm 4^a$
	HR (bpm)	356 ± 14	427 ± 16	$+21 \pm 4^a$	370 ± 12	-14 ± 3^a
Propranolol	MAP (mm Hg)	117 ± 3	111 ± 5	-5 ± 4	141 ± 6	$+26 \pm 5^a$
	HR (bpm)	347 ± 12	293 ± 12	-16 ± 4^a	274 ± 11	-7 ± 1^a

MAP, mean arterial blood pressure; HR, heart rate.
There were six rats in the saline-treated (0.9% NaCl, w/v) group. There were six rats in the methyl-atropine-treated (1 mg/kg, iv) group. There were seven rats in the propranolol-treated (1 mg/kg, iv) group.
The dose of N^G-nitro-L-arginine methyl ester (L-NAME) was 50 μmol/kg, iv.
Each value is the mean ± S.E.M. of the actual data or the percent changes in these parameters.
[a] $P < 0.05$, significant change.

Table 2
Cardiovascular effects of sodium nitroprusside, isoproterenol or 8-(4-chlorophenylthiol)-cAMP in saline- or L-NAME-treated rats

Compound	Parameter	Arithmetic change		%Change	
		Post-saline	Post-L-NAME	Post-saline	Post-L-NAME
SNP	ΔMAP (mm Hg)	-39 ± 7^{a}	-56 ± 8^{a}	-40 ± 7^{a}	-41 ± 6^{a}
	ΔHR (bpm)	$+8 \pm 1^{a}$	$+49 \pm 8^{a,b}$	$+2.0 \pm 0.3^{a}$	$+15 \pm 2^{a,b}$
Isoproterenol	ΔMAP (mm Hg)	-35 ± 3^{a}	-37 ± 5^{a}	-31 ± 3^{a}	$-24 \pm 6^{a,b}$
	ΔHR (bpm)	$+78 \pm 5^{a}$	$+112 \pm 8^{a,b}$	$+21 \pm 2^{a}$	$+36 \pm 3^{a,b}$
8-CPT-cAMP	ΔMAP (mm Hg)	-22 ± 3^{a}	$-38 \pm 4^{a,b}$	-18 ± 3^{a}	-23 ± 2^{a}
	ΔHR (bpm)	$+75 \pm 6^{a}$	$+123 \pm 12^{a,b}$	$+22 \pm 2^{a}$	$+42 \pm 6^{a,b}$

MAP, mean arterial blood pressure; HR, heart rate.
The dose of sodium nitroprusside (SNP) was 5 μg/kg, iv. The dose of isoproterenol was 0.5 μg/kg, iv. The dose of 8-(4-chlorophenylthiol)-cAMP (8-CPT-cAMP) was 10 μmol/kg, iv. The dose of N^{G}-nitro-L-arginine methyl ester (L-NAME) was 50 μmol/kg, iv.
In the sodium nitroprusside studies, the saline- and L-NAME-treated groups consisted of seven rats each. In the isoproterenol and 8-CPT-cAMP studies, the saline- and L-NAME-treated groups consisted of five rats each.
The values represent the mean ± S.E.M. of the arithmetic and percent changes in these parameters.
[a] $P < 0.05$, significant response.
[b] $P < 0.05$, post-L-NAME vs. post-saline.

in comparison to pre-L-NAME values). The sodium nitroprusside-induced falls in mean arterial blood pressure in the saline- and L-NAME-treated rats were not affected by the subsequent administration of propranolol ($P > 0.05$ for both comparisons). However, propranolol eliminated the sodium nitroprusside-induced increases in heart rate in the saline- and L-NAME-treated rats. The sodium nitroprusside-induced increases in heart rate in the saline- and L-NAME-treated rats following the administration of propranolol were $-1 \pm 2\%$ and $-3 \pm 2\%$, respectively, ($P > 0.05$ for both responses).

3.3. The effects of isoproterenol on mean arterial blood pressure and heart rate in saline- or L-NAME-treated rats

The effects of isoproterenol (0.5 μg/kg, iv) on mean arterial blood pressure and heart rate of saline- or L-NAME-treated (50 μmol/kg, iv) rats are summarized in Table 2. The effects of saline and L-NAME on resting cardiovascular parameters were similar to those summarized in Table 1. Isoproterenol produced a depressor response and a tachycardia in saline-treated rats ($P < 0.05$ for both responses). This dose of isoproterenol produced a similar depressor response in L-NAME- and saline-treated rats ($P > 0.05$). However, the tachycardia produced by isoproterenol was greater than in saline-treated rats ($P < 0.05$ for both comparisons).

3.4. The effects of 8-(4-chlorophenylthiol)-cAMP on mean arterial blood pressure and heart rate in saline- or L-NAME-treated rats

The effects of 8-(4-chlorophenylthiol)-cAMP (10 μmol/kg, iv) on mean arterial blood pressure and heart rate of saline- or L-NAME-treated (50 μmol/kg, iv) rats are summarized in Table 2. The effects of saline and L-NAME on mean arterial blood pressure and heart rate were similar to those shown in Table 1. 8-(4-chlorophenylthiol)-cAMP produced a depressor response and an increase in heart rate in saline-treated rats ($P < 0.05$ for both responses). The cAMP analogue produced a similar depressor response in L-NAME-treated as compared to saline-treated rats ($P > 0.05$). However, the tachycardia produced by 8-(4-chlorophenylthiol)-cAMP was greater than in saline-treated rats ($P < 0.05$).

3.5. The effects of tyramine on mean arterial blood pressure and heart rate of rats pretreated with saline, propranolol or L-NAME

The effects of tyramine (50 μg/kg, iv) on mean arterial blood pressure and heart rate of rats treated with saline, propranolol (1 mg/kg, iv) or L-NAME (50 μmol/kg, iv) are summarized in Table 3. The effects of saline, propranolol and L-NAME on resting mean arterial blood pressure and heart rate values were similar to those summarized in Table 1. Tyramine produced a pressor response and a tachycardia in saline-treated rats ($P > 0.05$ for both responses). Tyramine did not produce a tachycardia in propranolol-treated rats ($P > 0.05$) but produced a pressor response which was similar to that in saline-treated rats ($P > 0.05$). Tyramine produced an exaggerated increase in heart rate in L-NAME-treated rats. The arithmetic and percent changes in heart rate were greater than those observed in saline-treated rats ($P < 0.05$ for both comparisons). Tyramine also produced an arithmetically greater increase in mean arterial blood pressure in the L-NAME as compared to the saline-treated rats ($P < 0.05$). However, because of the elevated baseline mean arterial blood pressure values in L-NAME-treated rats, the percent change in

Table 3
Effects of tyramine on mean arterial blood pressure and heart rate in rats treated with saline, propranolol or L-NAME

Treatment	*N*	Parameter	Arithmetic change		%Change	
			Pre	Post	Pre	Post
Saline	5	ΔMAP (mm Hg)	+21 ± 2	+23 ± 3	+18 ± 3	+20 ± 4[a]
		ΔHR (bpm)	+52 ± 6	+61 ± 8	+15 ± 3	+18 ± 4
Propranolol	6	ΔMAP (mm Hg)	+22 ± 3	+17 ± 2	+19 ± 3	+17 ± 3
		ΔHR (bpm)	+59 ± 7	+7 ± 3[a]	+17 ± 3	+2 ± 2[a]
L-NAME	7	ΔMAP (mm Hg)	+22 ± 3	+32 ± 4[a]	+18 ± 3	+22 ± 3
		ΔHR (bpm)	+54 ± 7	+108 ± 11[a]	+16 ± 3	+37 ± 5[a]

MAP, mean arterial blood pressure; HR, heart rate; *N*, number of rats.
The dose of tyramine was 50 μg/kg, iv. The dose of propranolol was 1 mg/kg, iv. The dose of N^G-nitro-L-arginine methyl ester (L-NAME) was 50 μmol/kg, iv.
Each value represents the mean ± S.E.M. of the percent changes in these parameters.
[a] $P < 0.05$, post-treatment vs. pre.

mean arterial blood pressure produced by tyramine was similar to that in saline-treated rats ($P > 0.05$).

4. Discussion

The systemic administration of the NO synthesis inhibitor, L-NAME, produced an increase in mean arterial blood pressure in pentobarbital-anesthetized rats which was associated with a bradycardia. This bradycardia was not affected by the muscarinic receptor antagonist, methyl-atropine. This suggests that the bradycardia produced by L-NAME was due mainly to the withdrawal of sympathetic drive. These findings are consistent with those of Wang and Pang (1991) who provided evidence that the bradycardia produced by N^G-nitro-L-arginine in conscious rats was due mainly to an inhibition of sympathetic nerve activity, although there is evidence that the bradycardia produced by NO synthesis inhibitors involves an increase in cardiovagal drive (Widdop et al., 1992). The bradycardia associated with the pressor effects of L-NAME may be due to baroreceptor reflex-mediated changes in autonomic nerve activity to the heart. However, the results with sodium nitroprusside (see below) suggest that the baroreceptor heart rate reflex is markedly compromised in these pentobarbital-anesthetized rats. This raises the possibility that the L-NAME-induced bradycardia may be independent of the baroreceptor reflex. It is possible that L-NAME may reduce heart rate by direct actions in the brain or by direct actions on the autonomic nerves themselves. Although Buxton et al. (1993) provided evidence that L-NAME may be a muscarinic receptor antagonist, Han et al. (1994) found that L-NAME did not block muscarinic receptors in isolated cardiac pacemaker cells. As such, systemically-injected L-NAME may not directly antagonize muscarinic receptors in the heart of pentobarbital-anesthetized rats.

The administration of the NO-donor, sodium nitroprusside, produced a pronounced depressor response but only a minor tachycardia. This suggests that the baroreceptor reflex is markedly compromised in these pentobarbital-anesthetized rats. Moreover, it suggests that the pronounced increases in heart rate produced by isoproterenol and 8-(4-chlorophenylthiol)-cAMP are due to the direct actions of these agents on the heart. However, the sodium nitroprusside-induced depressor responses were associated with a much larger tachycardia in L-NAME-treated than in saline-treated rats. The sodium nitroprusside-induced increases in heart rate in these rats were abolished by the β-adrenoceptor antagonist, propranolol. This suggests that these increases in heart rate were due mainly to the activation of cardiac sympathetic drive. The augmented tachycardia produced by sodium nitroprusside in the L-NAME-treated rats may be due to, (i) augmented release of norepinephrine from sympathetic nerve terminals, (ii) the up-regulation of β-adrenoceptor signal transduction processes in response to diminished release of norepinephrine from cardiac sympathetic nerves, and (iii) up-regulation of β-adrenoceptor signal transduction processes due to the inhibition of NO synthesis in cardiac pacemaker cells (see below).

β-Adrenoceptor agonists increase pacemaker rate by alteration of ion-channel activity including the cAMP-mediated activation of voltage-sensitive L-type Ca^{2+}-channels (Han et al., 1994). NO synthase is present in cardiac nerve fibers and intrinsic neurons of the rat and guinea pig heart (Klimaschewsi et al., 1992), in cardiac pacemaker cells (Han et al., 1994) and in cardiac endothelium (endocardium) and in myocytes (Schulz et al., 1992; Balligand et al., 1995). The increase in intracellular Ca^{2+} resulting from activation of voltage-sensitive L-type Ca^{2+}-channels would stimulate NO synthase activity in cardiac tissues (Schulz et al., 1992; Han et al., 1994). NO generated within isolated cardiac pacemaker cells markedly diminishes the chronotropic effects of β-adrenoceptor agonists via increases in cGMP (Hare et al., 1995). Moreover, there is considerable evidence that intracellular cGMP counteracts the effects of cAMP in the heart (Hare et al., 1995).

The present study demonstrates that the isoproterenol-induced depressor responses was unaffected by L-NAME whereas the tachycardia was augmented. This latter finding

provides further evidence that cardiac derived NO suppresses the positive chronotropic and inotropic effects produced by stimulation of β_1-adrenoceptors (Hayes et al., 1984; Han et al., 1994). The augmented tachycardia to isoproterenol may be due to the loss of the inhibitory influence of NO on the β-adrenoceptor-mediated activation of Ca^{2+}_{VSL}-channels (Han et al., 1994). As such, it is possible that the withdrawal of cardiac sympathetic drive may indirectly diminish NO synthesis by reducing the influx of Ca^{2+} into cardiac pacemaker cells. Moreover, the resultant loss of cGMP would lead to the enhancement of cAMP signaling. The mechanisms by which a diminution of cGMP levels augments cAMP signaling may involve (i) diminished degradation of cAMP by phosphodiesterases (Bourne et al., 1973), (ii) diminished expulsion of cAMP from cells (Brunton and Mayer, 1979), or (iii) enhanced activity of cAMP-dependent protein kinase (Hausdorff et al., 1990; Palczewski and Benovic, 1991; Koch et al., 1995; Premont et al., 1995). The reduction in cardiac sympathetic nerve activity produced by L-NAME would diminish β-adrenoceptor-mediated activation of cAMP-dependent protein kinase and G protein-coupled receptor kinases (Hausdorff et al., 1990; Palczewski and Benovic, 1991; Koch et al., 1995; Premont et al., 1995). These protein kinases desensitize β-adrenoceptors by phosphorylating these proteins and thereby preventing the receptor from associating with G_s proteins. The phosphorylated receptor is then sequestered into specialized organelles where it is dephosphorylated prior to re-incorporation into the plasma membrane (Hausdorff et al., 1990). As such, the loss of norepinephrine-mediated stimulation of β_1-adrenoceptors after administration of L-NAME may lead to an increase in the affinity and/or density of cardiac β_1-adrenoceptors. These changes would facilitate the responses to endogenous or exogenously applied β_1-adrenoceptor agonists.

The 8-(4-chlorophenylthiol)-cAMP-induced depressor response was unaffected whereas the tachycardia was augmented in the presence of L-NAME. This finding supports evidence that cGMP inhibits cAMP signaling in pacemaker cells and cardiac myocytes (Hayes et al., 1984; Han et al., 1994). The blockade of cardiac β-adrenoceptors augments the tachycardia produced by 8-(4-chlorophenylthiol)-cAMP in pentobarbital-anesthetized rats (Whalen et al., 1998). This suggests that the activation of β-adrenoceptors regulates the potency of cAMP within the heart (Whalen et al., 1998). Accordingly, the augmented tachycardia produced by 8-(4-chlorophenylthiol)-cAMP and isoproterenol in L-NAME-treated rats may be due to the direct enhancement of the chronotropic effects of intracellular cAMP.

Neurotransmitter stores of norepinephrine in sympathetic nerve terminals are localized within vesicles subject to Ca^{2+}-dependent exocytosis and within cytoplasmic-protected pools which are released in a Ca^{2+}-independent process by ischemia (Schömig et al., 1987, 1988). Tyramine is selectively taken up by post-ganglionic sympathetic nerve terminals because it is a substrate for catecholamine-uptake$_1$ processes in these terminals (Bonisch, 1986; Langeloh and Trendelenburg, 1987; Langeloh et al., 1987). Upon entry, tyramine induces the non-exocytotic outward transport of cytoplasmic-protected pools of norepinephrine by Ca^{2+}-independent processes but does not mobilize norepinephrine-containing vesicles which co-localize adenosine triphosphate and neuropeptide Y (Bonisch, 1986; Langeloh and Trendelenburg, 1987; Langeloh et al., 1987). The arithmetic changes in mean arterial blood pressure produced by tyramine were augmented in L-NAME-treated rats although the percent changes were similar because of the L-NAME-induced increase in baseline mean arterial blood pressure. The tyramine-induced tachycardia was substantially greater in rats treated with L-NAME. The facilitated tachycardia may involve the enhanced release of norepinephrine from sympathetic nerve terminals since NO inhibits the release of norepinephrine from post-ganglionic sympathetic nerve terminals (Greenberg et al., 1990). To our knowledge, there is no direct evidence that inhibition of NO synthesis facilitates norepinephrine release from cardiac sympathetic nerve terminals, although NO derived from cardiac cells (Klimaschewsi et al., 1992; Schulz et al., 1992; Han et al., 1994; Balligand et al., 1995) may inhibit norepinephrine release from these nerve terminals.

In summary, the systemic administration of L-NAME enhances the tachycardia produced by sympathetically-derived norepinephrine and exogenously administered isoproterenol. This may be due to the direct blockade of NO synthase activity by L-NAME as well as the ability of this compound to indirectly block NO synthase activity by a reduction in cardiac sympathetic nerve activity. The exaggerated effects of β_1-adrenoceptor stimulation may therefore be due to the loss of NO-mediated cGMP-dependent inhibition of cAMP signaling although it is possible that an up-regulation of cardiac β_1-adrenoceptor is also involved. These findings may explain why the centrally-mediated activation of cardiac sympathetic drive produces an exaggerated tachycardia in conscious L-NAME-treated rats (Davisson et al., 1994).

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