Uncoupling of the Baroreflex by N\textsubscript{N}-Cholinergic Blockade in Dissecting the Components of Cardiovascular Regulation

John R. Shannon, Jens Jordan, Bonnie K. Black, Fernando Costa, David Robertson

Abstract—Systemic administration of adrenergic agonists and nitric oxide donors is used extensively to determine cardiovascular receptor sensitivity. Conclusions regarding receptor sensitivity in the presence of the baroreflex may be misleading. In 8 normal volunteers, we determined the heart rate and blood pressure changes after incremental bolus doses of isoproterenol, phenylephrine, and sodium nitroprusside before and during neuronal nicotinic cholinergic (N\textsubscript{N}-cholinergic) blockade with trimethaphan. Results are given as median (25th/75th percentile). With trimethaphan, the baroreflex slope (as determined by bolus doses of nitroprusside and phenylephrine) decreased from 24 (22/26) to 0.00 (0.00/0.09) mmHg (P<0.01). The dose of isoproterenol that decreased systolic blood pressure (SBP) 12.5 mmHg changed from 0.61 (0.51/5.3) to 0.17 (0.12/0.21) µg (P<0.01); the dose required to increase heart rate 12.5 bpm changed from 0.22 (0.17/0.41) to 0.74 (0.33/2.3) µg (P<0.01). The dose of nitroprusside required to decrease SBP 12.5 mmHg changed from 2.3 (1.3/3.4) to 0.18 (0.14/0.24) µg/kg (P<0.01). The dose of phenylephrine required to increase SBP 12.5 mmHg changed from 135 (110/200) to 16 (10/30) µg (P<0.01). We conclude that the efferent arc of the baroreflex can be completely interrupted with N\textsubscript{N}-cholinergic blockade. Estimation of adrenoreceptor sensitivity and sensitivity to nitric oxide donors by systemic administration of agonists is severely confounded by baroreflexes. Uncoupling of the baroreflex by N\textsubscript{N}-cholinergic blockade may be a useful method to obtain an integrated measure of adrenergic receptor sensitivity and sensitivity to nitric oxide donors in humans. This approach would permit the comparison of normal and abnormal physiological states without the “noise” of baroreflex buffering. (Hypertension. 1998;32:101-107.)

Key Words: receptors, adrenergic ■ phenylephrine ■ nitroprusside ■ isoproterenol ■ trimethaphan

Systemic administration of adrenergic agonists is used extensively to determine adrenoreceptor sensitivity in humans.\textsuperscript{1,2} Similarly, the influence of the distal part of the nitric oxide pathway on cardiovascular responses can be evaluated by systemic administration of nitric oxide donors. A possible role of changes in adrenergic sensitivity or sensitivity to nitric oxide donors in a variety of human conditions has been postulated based on these methods.\textsuperscript{2,3} Results of these studies, however, may be confounded by baroreflex-mediated alterations of sympathetic and parasympathetic tone.\textsuperscript{4,5} In a few studies, an effort was made to block baroreflex-mediated changes of HR and BP with atropine alone\textsuperscript{1,3} or atropine in combination with a centrally acting sympatholytic drug.\textsuperscript{4} The main limitation of these studies is that either the sympathetic outflow was not blocked\textsuperscript{1,3} or was only partially blocked\textsuperscript{4}; or that the completeness of the blockade was not assessed. Therefore, the influence of the baroreflex on the cardiovascular responses cannot be inferred reliably. The efferent arc of the baroreflex (parasympathetic and sympathetic nerves) can be blocked by N\textsubscript{N}-cholinergic antagonists\textsuperscript{10} commonly referred to as ganglionic blockers.\textsuperscript{11} It has been shown recently that N\textsubscript{N}-cholinergic blockade can be used to attenuate the influence of the baroreflex on systemic cardiovascular responses to drugs.\textsuperscript{5}

The purpose of this study was to determine whether or not the efferent arc of the baroreflex could be interrupted completely by N\textsubscript{N}-cholinergic blockade in human subjects. Furthermore, we evaluated the sensitivity to sodium nitroprusside (nitric oxide donor), phenylephrine (\(\alpha\)-adrenoreceptor agonist), and isoproterenol (\(\beta\)\textsubscript{1}- and \(\beta\)\textsubscript{2}-adrenoreceptor agonist) before and after complete blockade of the efferent arc of the baroreflex.

Methods

Subjects

Eight healthy subjects (4 male, 4 female) were recruited from a pool of normal volunteers. Median (25th/75th percentile) age, weight, and height were 30 (25/36) years, 64 (60/88) kg, and 170 (164/180) cm, respectively. All subjects underwent a thorough clinical examination, ECG, and admission urinalysis and blood work. Written informed consent was obtained before study entry. All studies were approved by the institutional review board.

Protocol

Four days before study, volunteers were placed on a 150 mEq Na\textsuperscript{+} and 70 mEq K\textsuperscript{+} diet free of substances that could interfere with catecholamine measurements. All vasoactive medications were discontinued at least 5 half-lives before testing. The volunteers were admitted to the Elliot V. Newman Clinical Research Center at...
Interval. Log dose-response curves were determined for the change at 0.05
25 mm Hg. Incremental bolus doses of sodium nitroprusside starting
given to increase HR by at least 25 bpm or to decrease SBP by
12.5 mm Hg were determined by interpolation from the regression
of 50
increase SBP by 25 mm Hg were administered, starting with a dose
Similarly, incremental bolus doses of phenylephrine sufficient to
decrease SBP by 25 mm Hg or increase HR at least 25 bpm.

Manual brachial BP readings were obtained at baseline and repeat-
edly during testing. To insure accuracy of beat-to-beat BP measure-
ments, the pulse pressure from the Finapres recording was adjusted
for the average of at least 3 consecutive simultaneously determined
beat-to-beat by photoplethysmography (Finapres, Ohmeda 2300).

Criteria for an adequate MSNA recording were as follows: (1)
afferent signals, whereas cutaneous stimulation by slight stroking of
the skin did not; (3) the neurogram showed typical morphology; and
(4) nerve traffic during phase II of the Valsalva maneuver increased.

Pharmacological Testing
Pharmacological testing was conducted with subjects in the recumbent
position at least 2.5 hours after their last meal. HR was
determined with continuous ECG, and BP changes were measured
beat to beat by photoplethysmography (Finapres, Ohmeda 2300).

The infusion rate of trimethaphan necessary to completely
block the efferent arc of the baroreflex in our subjects was 6
(6/7) mg/min [0.1 (0.07/0.1) mg · min⁻¹ · kg⁻¹]. When the
infusion was started at 6 mg/min, complete blockade of the
eff erent arc of the baroreflex was attained after approximately
10 to 15 minutes. This infusion rate was well tolerated in all
subjects, and we did not observe any respiratory complica-
tions, although 1 subject reported a sensation of dyspnea at
an infusion rate of 6 mg/min. The infusion was stopped for
approximately 2 minutes until symptoms resolved and re-
sumed at 5 mg/min, which was well tolerated for the
remainder of the study.

SBP was 118 (114/123) mm Hg at baseline and 107
(101/123) mm Hg during trimethaphan infusion (P<0.08).
Diastolic blood pressure was 77 (63/85) mm Hg at baseline
and 70 (67/88) mm Hg during trimethaphan infusion
(P=NS). HR increased from 59 (54/66) to 86 (81/89)
(P<0.01). The HR and BP variability was markedly attenu-
ated (Figure 1). With Nₐ-cholinergic blockade, there was a
marked decrease in plasma norepinephrine and a concomitant
decrease in the plasma dihydroxyphenyl(ethylene)glycol and
dihydroxyphenylacetic acid, products of the intraneuronal
metabolism of norepinephrine and dopamine, respectively.
There was no significant change in plasma epinephrine
(Table).

The HR and SBP responses to a bolus dose of phenyleph-
rine before and during trimethaphan infusion in a represen-
tative subject are illustrated in Figure 2. There was no
compensatory change in HR despite a 23-mm Hg increase in
SBP. The baroreflex slope, as determined by administration
of phenylephrine and nitroprusside, decreased from 24 (22/
26) ms/mm Hg at baseline to 0.00 (0.00/0.09) ms/mm Hg
with trimethaphan (P<0.01). This effect of trimethaphan was
sustained throughout the study. The combined baroslopes of all subjects before and during N,N-cholinergic blockade are illustrated in Figure 3.

MSNA was completely eliminated with an infusion of trimethaphan at 6 mg/min (Figure 4). Within 20 minutes after discontinuation of the trimethaphan infusion, bursts of MSNA reappeared. Before trimethaphan infusion, there was a dose-dependent increase and decrease in MSNA with nitroprusside and phenylephrine, respectively. During trimethaphan infusion, there were no changes in MSNA with either nitroprusside (decrease of SBP by 30 mm Hg) or phenylephrine (increase of SBP by 30 mm Hg).

The tachycardic effect of isoproterenol was greatly attenuated with N,N-cholinergic blockade. During trimethaphan infusion, the dose of isoproterenol required to increase HR 12.5 bpm changed from 0.22 (0.17/0.41) µg to 0.74 (0.33/2.3) µg. In fact, in some subjects, there was almost no change in HR with isoproterenol during N,N-cholinergic blockade. In contrast, there was a large increase in the sensitivity to the hypotensive effect of isoproterenol (Figure 5). The dose of isoproterenol that decreased SBP 12.5 mm Hg decreased from 0.61 (0.51/5.3) to 0.17 (0.12/0.21) µg (Figure 6) (P<0.01).

| Plasma Catecholamines Before (Baseline) and During (Trimethaphan) N,N-Cholinergic Blockade |
|---------------------------------|-----------------|-----------------|
| Norepinephrine                  | 0.83 (0.63/1.9) | 0.26 (0.18/0.37) | <0.01 |
| Epinephrine                     | 0.11 (0.076/0.22) | 0.076 (0.022/0.131) | NS |
| DHPG                            | 6.4 (5.2/7.6) | 4.3 (3.9/6.3) | <0.05 |
| DOPAC                           | 16 (11/19) | 11 (6.3/16) | <0.05 |

DHPG indicates dihydroxyphenyl(ethylene)glycol; DOPAC, dihydroxyphenylacetic acid.

Plasma catecholamines were obtained at baseline after at least 30 minutes supine and approximately 20 minutes after the trimethaphan infusion was begun (NS= P>0.05). Values are median (25th/75th percentile).

The dose of nitroprusside required to decrease SBP 12.5 mm Hg decreased about 13-fold, from 2.3 (1.3/3.4) µg/kg before N,N-cholinergic blockade to 0.18 (0.14/0.24) µg/kg with trimethaphan (P<0.01) (Figure 6).
phenylephrine required to increase SBP 12.5 mm Hg decreased 8-fold, from 135 (110/200) mg to 16 (9.7/30) mg (P < 0.01) (Figure 6).

The sensitivity to nitroprusside, isoproterenol, and phenylephrine with N\textsubscript{N}-cholinergic blockade could not be predicted from plasma norepinephrine levels achieved with N\textsubscript{N}-cholinergic blockade.

Discussion

The main finding of this study is that the efferent arc of the baroreflex can be interrupted completely by readily achievable levels of N\textsubscript{N}-cholinergic blockade in human subjects. After interruption of the efferent arc of the baroreflex, there was a dramatic change in the cardiovascular responses to the systemic administration of sodium nitroprusside, phenylephrine, and isoproterenol.

Ganglionic blockers interrupt sympathetic and parasympathetic nerve traffic by competitively binding to postsynaptic N\textsubscript{N}-cholinergic receptors of autonomic ganglia. \textsuperscript{11,16} By contrast, cholinergic agonism increases sympathetic and parasympathetic outflow. \textsuperscript{17} Trimethaphan, a nondepolarizing N\textsubscript{N}-cholinergic antagonist, has been extensively used for the acute treatment of arterial hypertension.\textsuperscript{18–20} In contrast to depolarizing agents, it causes no initial stimulation of the postganglionic neuron. Acetylcholine release from preganglionic neurons is not affected.\textsuperscript{21} Trimethaphan is known to cause histamine release under some experimental conditions.\textsuperscript{22} With continuous infusion, plasma histamine levels initially increase but return to baseline after approximately 10 minutes and appear not to contribute to the hypotensive effect in humans.\textsuperscript{22} The concentration of trimethaphan necessary to achieve a direct vasodilatory effect in vitro is approximately 10 to 100 times greater than the concentration necessary to achieve ganglionic blockade.\textsuperscript{23,24}

With interruption of the efferent arc of the baroreflex, homeostatic adjustments normally controlled by the autonomic nervous system (BP, HR, sweating, and other factors) are blunted or abolished. A similar interruption of the efferent arc of the baroreflex occurs in autonomic failure. Thus, N\textsubscript{N}-cholinergic blockade mimics the clinical picture seen in...
patients with pure autonomic failure and multiple system atrophy have been shown to have markedly increased sensitivity to the depressor effect of \( \beta_2 \)-agonists and to the pressor effect of \( \alpha_1 \)-agonists and sympathomimetics. In fact, local administration of \( \alpha_1 \)-agonists (eg, eye drops) can cause marked increases of blood pressure in such patients. Patients with primary autonomic failure also have hypersensitivity to the depressor effect of nitroglycerin.

The afferent arc of the baroreflex can also be interrupted as a complication of extensive neck surgery or irradiation. Patients with bilateral interruption of the afferent baroreflex arc (baroreflex failure) present with paroxysms of severe hypertension and tachycardia resembling the clinical manifestations of pheochromocytoma. In addition, these patients may have episodes of hypotension and bradycardia. The levels of BP and HR depend more on level of arousal rather than on posture. In spite of clinical presentations vastly different from that of primary autonomic failure, patients with baroreflex failure also have a several-fold increase in sensitivity to the pressor response of \( \alpha_1 \)-agonists and to the depressor effects of nitric oxide donors.

Changes of central sympathetic or parasympathetic outflow may also change baroreflex sensitivity. Therefore, with disruption of either the afferent or efferent arc of the baroreflex, or with changes in central sympathetic or parasympathetic outflow, the sensitivity to vasoactive agents is significantly altered. Considering the large changes in response to vasoactive agents with complete disruption of the baroreflex arc, even small changes in the sensitivity of the baroreflex could significantly confound the interpretation of cardiovascular responses. Many disorders that may have changes in adrenergic receptor sensitivity or nitric oxide metabolism have also been shown to have changes in baroreflex function. Even commonly used medications (eg, digoxin) and dietary substances (eg, caffeine) may affect baroreflex function. Furthermore, in normal subjects, the interindividual variability of baroreflex sensitivity appears to be relatively large.

The influence of the baroreflex might limit the usefulness of some commonly employed approaches used to assess adrenergic receptor sensitivity. Local application of vasoactive agents (eg, forearm blood flow model) are sometimes employed to limit baroreflex activation. Theoretically, however, even the small amounts of drug reaching the systemic circulation may influence sympathetic and parasympathetic tone.

One possible approach to address receptor sensitivity in humans is to determine responses to cardiovascular drugs after pharmacological interruption of the efferent arc of the baroreflex. The decrease in sinus arrhythmia with atropine simplifies HR determination. Atropine does not block sympathetic outflow to the vasculature and the heart. Ford and James used atropine and clonidine to block parasympathetic effects and inhibit central sympathetic outflow. Clonidine, however, attenuates rather than eliminates sympathetic outflow. Furthermore, clonidine has peripheral effects that may influence BP regulation. Recently it has been shown that \( \beta_2 \)-cholinergic block can be used to interrupt the baroreflex in human subjects. We and others have shown that complete interruption of the baroreflex arc can be achieved with this method. The lack of HR changes with either decreases or increases in BP strongly suggests complete ganglionic blockade. Theoretically, there could be small changes in sympathetic and parasympathetic nerve traffic not reflected in HR changes. We demonstrated that trimethaphan completely blocked MSNA and that this blockade could not be overcome by activation of the baroreflex (nitroprusside bolus).

With interruption of the baroreflex, cardiovascular responses to drugs can be observed in the absence of compensatory changes of sympathetic and parasympathetic tone. In this study, there was a significant decrease in the chronotropic response to isoproterenol after \( \beta_2 \)-cholinergic blockade. By contrast, autonomic failure patients have been shown to have an increase in the chronotropic response to isoproterenol. These disparate observations with chronic (autonomic failure) and acute (\( \beta_2 \)-cholinergic blockade) interruption of the efferent arc of the baroreflex may suggest an increase in \( \beta_2 \)-adrenoreceptor sensitivity or number over time. The depressor effect of isoproterenol was markedly augmented with \( \beta_2 \)-cholinergic blockade, reaching the sensitivity observed in autonomic failure patients. The changes in cardiovascular responses to isoproterenol during \( \beta_2 \)-cholinergic blockade suggest that the increase in HR seen with isoproterenol in the absence of blockade is greatly influenced by the indirect or baroreflex-mediated effect on \( \beta_2 \)-adrenoceptors and that direct stimulation of cardiac \( \beta_2 \)-adrenoceptors is less important. The upper end of the dose range of the isoproterenol HR response could not be fully explored during \( \beta_2 \)-cholin-
ergic blockade given the powerful vasodepressor effect of the drug in the absence of the baroreflex. Paradoxically, there is an increase of BP with continuous infusion of isoproterenol but a decrease of BP with bolus doses of isoproterenol. The increase of BP with continuous infusion of isoproterenol may be due to β1-mediated renin release or β2-mediated release of catecholamines from postganglionic adrenergic nerve endings. Therefore, bolus administration of isoproterenol after Nα-cholinergic blockade may be more useful to obtain an integrated measure of vascular β1 sensitivity than continuous infusion.

After Nα-cholinergic blockade, we observed a 10-fold increase in sensitivity to the pressor effect of phenylephrine. It has previously been observed that the pressor effect of norepinephrine and angiotensin II is augmented with the use of ganglionic blockers. Interruption of the baroreflex arc with Nα-cholinergic blockade seems to be the most likely explanation for the pressor hypersensitivity. We observed the change in the pressor response to phenylephrine immediately after Nα-cholinergic blockade (5 to 10 minutes), and the magnitude of this pressor response remained stable throughout the study (1 to 2 hours). Furthermore, there was no relation between plasma norepinephrine level and the pressor effect of phenylephrine with Nα-cholinergic blockade. Therefore, it appears unlikely that the increase in the pressor response so soon after initiation of Nα-cholinergic blockade would be related to upregulation of adrenoreceptors. The sensitivity to phenylephrine after Nα-cholinergic blockade is comparable to sensitivities observed in severe autonomic failure and baroreflex failure. Much of the hypersensitivity to pressor agents in autonomic failure has been interpreted to be due to upregulation of adrenoreceptors in the setting of low circulating catecholamines. However, debuffering of the baroreflex alone could account for most of the pressor hypersensitivity in autonomic failure. In the complete absence of norepinephrine and epinephrine, one would expect extreme upregulation of α-adrenoreceptors. It has been shown, however, that patients with dopamine β-hydroxylase deficiency, who lack the ability to synthesize norepinephrine and epinephrine but still have an intact baroreflex at least in terms of parasympathetic activation, have less hypersensitivity to α-agonists than patients with primary autonomic failure. Another possible reason for the increase in the sensitivity to adrenergic agonists could be decreased release of norepinephrine from nerve terminals. Decreased release of norepinephrine may leave morepostsynaptic receptors uncoupled, which could be available for binding to a systemically administered agonist. This explanation is not supported by the lack of a relation between plasma norepinephrine and sensitivity to phenylephrine (and isoproterenol) observed in this study.

The large increase in the depressor effect of nitroprusside during Nα-cholinergic blockade indicates that the cardiovascular effect of nitric oxide donors is buffered by the baroreflex. In some subjects there was an increase in the HR but only a very small change of BP with nitroprusside before trimethaphan. It has been shown in animals that nitric oxide has central nervous effects and decreases sympathetic outflow. A similar central nervous effect of nitric oxide in humans may be suggested by its bradycardic effect in patients with interruption of low-pressure baroreceptor transmission (cardiac transplant) and in patients with baroreflex failure and integrity of the efferent vagal innervation of the heart (selective baroreflex failure). We did not observe a bradycardia with nitroprusside after interruption of the efferent arc of the baroreflex with Nα-cholinergic blockade. Uncoupling of the baroreflex by Nα-cholinergic blockade may be a useful method for obtaining an integrated measure of adrenergic receptor sensitivity and sensitivity to nitric oxide donors in humans. This approach would permit the comparison of normal and abnormal physiological states without the “noise” of baroreflex buffering.

**Acknowledgments**

This study was supported in part by National Institutes of Health grants RR00095 and NS33460 and by NASA grants NAS 9–19483 and NAGW3873. Jens Jordan is supported by the Deutsche Forschungsgemeinschaft.

**References**


Uncoupling of the Baroreflex by N\textsubscript{N}-Cholinergic Blockade in Dissecting the Components of Cardiovascular Regulation

John R. Shannon, Jens Jordan, Bonnie K. Black, Fernando Costa and David Robertson

_Hypertension_. 1998;32:101-107
doi: 10.1161/01.HYP.32.1.101

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/32/1/101