Persistence of Sympathetic-Mediated Forearm Vasoconstriction After α-Blockade in Hypertensive Patients

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Sympathetic vasoconstriction not mediated by α-adrenoceptors has been identified in vitro and in animals but not in humans. We evaluated the effect of α-adrenoceptor blockade on either endogenous vascular sympathetic activation (obtained through the application of a nonhypotensive lower-body negative pressure, −10 mm Hg for 5 minutes) or selective postsynaptic α-adrenoceptor stimulation by exogenous norepinephrine (0.005 μg/100 ml forearm tissue/min for 3 minutes) in the presence of β-blockade by propranolol (10 μg/100 ml forearm tissue/min for 15 minutes). Drugs were infused into the brachial artery at systemically ineffective rates while continuously monitoring forearm blood flow (by venous plethysmography), intra-arterial mean arterial pressure, and heart rate in patients with essential hypertension. The irreversible antagonist phenoxybenzamine was used at a rate of 20 μg/100 ml forearm tissue/min for 1 hour, which antagonized the local responses to norepinephrine in a range of 0.005–0.05 μg/100 ml forearm tissue/min. During saline administration, either lower-body negative pressure or exogenous norepinephrine decreased forearm blood flow comparably. However, after phenoxybenzamine administration, forearm vasoconstriction to norepinephrine was abolished while a residual response to lower-body negative pressure remained in each patient. To exclude insufficient α-adrenoceptor blockade, the same experimental protocol was repeated by doubling phenoxybenzamine concentrations. No difference from the data obtained with the lower level of antagonist was found. Further studies were performed to confirm the sympathetic origin of the residual vasoconstriction. Bretylium tosylate, a neurotransmitter blocker, infused into the brachial artery (50 μg/100 ml forearm tissue/min for 90 minutes) abolished the effect of endogenous sympathetic activation but did not alter the effect of exogenous norepinephrine. The data are consistent with the presence of sympathetic vasoconstriction not mediated by α-adrenoceptors in forearm arterioles of patients with essential hypertension. ([Circulation 1989;80:485–490])

Results from several in vitro and animal studies contrast with the view of vascular α-adrenoceptors as the sole mediators of neural sympathetic stimuli.1–5 As a matter of fact, the now rather widely accepted concept of sympathetic vasoconstriction not mediated by α-adrenoceptors6 evolved from pharmacologic experiments in which α-adrenoceptor blockers, even at very high concentrations, could not abolish the effect of vasoconstriction mediated by sympathetic stimuli.6 However, no attempt has been made to replicate those studies in humans, at least to our knowledge.

To approach that problem, we obtained a standardized activation of sympathetic discharge by applying a nonhypotensive lower-body negative pressure (LBNP) and tested the effect of pharmacologic antagonists in patients with essential hypertension. To avoid confounding changes in systemic hemodynamics and reflexogenic activation of autonomic activity, drugs were infused into the brachial artery at systemically ineffective rates.

Methods

Subjects

Twenty-one patients (13 men, eight women; mean age, 43.3±7.9 years) with mild-to-moderate, uncomplicated essential hypertension (164.8±10.1/99.7±8.7 mm Hg) were recruited for the formal series of studies reported in this paper. According to institutional guidelines, all patients were aware of the investigational nature of the study and consented to it. All drugs were withdrawn for at least 1

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Received January 5, 1989; revision accepted April 18, 1989.
week before study, and no patient had been receiving medications that had a long-term effect on sympathetic neurotransmission.

Our experimental design accounted for the activation of endogenous sympathetic activity by LBNP. Its effects were compared with those of discrete \( \alpha \)-adrenoceptor stimulation mimicked by exogenous norepinephrine (NE). Effective local propranolol pretreatment (10 \( \mu \)g/100 ml forearm tissue/min for 15 minutes) avoided the confounding interferences from \( \beta \)-adrenoceptor stimulation\(^7\) during both maneuvers.

**Endogenous Sympathetic Activation**

Endogenous sympathetic activation was obtained by a –10 mm Hg LBNP (for 5 minutes), which reduces venous return, deactivates cardiopulmonary inhibitory pathways, and causes sympathetic discharge\(^8\) and peripheral vasoconstriction without changing systemic blood pressure and heart rate.\(^9\) Negative pressure was preliminarily titrated to obtain effects comparable to exogenous NE (see below).

**\( \alpha \)-Adrenoceptor Stimulation**

\( \alpha \)-Adrenoceptor stimulation was induced by intraarterial infusion of exogenous NE at a rate (0.005 \( \mu \)g/100 ml forearm tissue/min for 3 minutes) derived from the results of the phenoxybenzamine (PBZ) studies. Both stimuli were administered at time intervals adequate to provide steady baselines.

\( \alpha \)-Blockade was obtained by PBZ, whose irreversible alkylating effect avoided displacement from the receptor, at variance with other antagonists of a competitive nature.\(^10\) PBZ was continuously infused for 60 minutes, which is a period sufficient for the full development of its effects.\(^11,12\) The drug was administered at a rate of 20 \( \mu \)g/100 ml forearm tissue/min, which was chosen to approximate local arterial concentrations of those used in previous experimental studies.\(^10\) The resulting antagonism by PBZ was tested in five patients according to the changes in the vascular effect of three cumulative intra-arterial NE rates (0.005, 0.015, 0.05 \( \mu \)g/100 ml forearm tissue/min for 3 minutes each, in presence of propranolol). PBZ abolished forearm vasoconstriction to the first and second NE infusion rate and reduced by 80.5±11.1% the vasoconstriction to the third rate (Figure 1).

**Irreversible \( \alpha \)-Blockade by Phenoxybenzamine**

This series was designed to determine whether or not forearm vasoconstriction is resistant to \( \alpha \)-blockade.

Six hypertensive patients underwent either LBNP (–10 mm Hg for 5 minutes) or intra-arterial NE (0.005 \( \mu \)g/100 ml forearm tissue/min for 3 minutes) in the absence (saline, 0.201 ml/min) or presence of PBZ (20 \( \mu \)g/100 ml forearm tissue/min for 60 minutes). The same protocol was repeated in four additional patients by using a doubled infusion rate of PBZ (40 \( \mu \)g/100 ml forearm tissue/min).

**Neurotransmission Blockade by Bretylium**

In this series, we sought evidence for the sympathetic nature of LBNP-mediated vasoconstriction. In addition, we wanted to substantiate the assumption that an amount of drug sufficient to block neurotransmission could be delivered by the intraluminal route to the advential and outer mediascular layers where sympathetic endings prevail.\(^13\) Bretylium tosylate, a neurotransmitter blocker,\(^14\) was used for this purpose. As in the previous series, either LBNP (–10 mm Hg for 5 minutes) or intra-arterial NE (0.015 \( \mu \)g/100 ml forearm tissue/min for 3 minutes) (both in the presence of propranolol) were administered in the absence and presence of bretylium at 50 \( \mu \)g/100 ml forearm tissue/min for 90 minutes (six patients) according to previous human data\(^15\) validated in pilot studies.

**Experimental Procedure**

All studies were performed in a quiet climatized room. A polyethylene cannula (21 gauge, Abbott, Sligo, Ireland) was inserted into the left brachial artery under light local anesthesia (2% lidocaine), and it was connected through stopcocks to a pres-
FIGURE 2. Time course of forearm blood flow (FBF) during lower-body negative pressure (LBNP, −10 mm Hg for 5 minutes) and exogenous norepinephrine (NE, 0.005 μg/100 ml forearm tissue/min for 3 minutes) in the absence and presence of α-adrenoceptor blockade through phenoxybenzamine (20 μg/100 ml forearm tissue/min for 60 minutes). Data obtained in presence of propranolol (10 μg/100 ml forearm tissue/min for 15 minutes before the intervention). ○, experimental forearm; ●, contralateral forearm. Data are mean±SEM (n=6).

sure transducer (Model MS20, Electromedics, Englewood, Colorado) for systemic mean blood pressure (1/5 pulse pressure plus diastolic pressure), heart rate monitoring (Model VSM1, Physiocontrol, Redmond, Washington), and for intra-arterial infusions. The electronic beat-to-beat signal was digitized online through an AT-IBM compatible personal computer by using a customized software program (Sanweari SNC, Casalecchio sul Reno, Bologna, Italy). A strain-gauge plethysmograph (LOOSCO, GL LOOS, Amsterdam, The Netherlands) was used to measure forearm blood flow (FBF) at both limbs. Details concerning the sensitivity and the reproducibility of the method as performed in our laboratory have been published.

Determination of forearm volume was performed according to the water displacement method. Drug infusion rates were normalized for 1 dl of tissue by adjusting the speed of infusion to the desired infusion rates. Drugs were infused through separate ports with three-way stopcocks as needed.

For the LBNP experiments, the inferior limbs were placed up to the waist into an air tight, Plexiglas-made container, sealed by rubber flaps around the waist. Negative pressures, quantified by a U-shaped mercury manometer connected to the inside of the apparatus, were generated through a hoover vacuum (Bosch, Hamburg, FRG).

Statistical Analysis

Because no relevant changes in mean arterial pressure occurred during the study, all data were analyzed in terms of FBF. Wilcoxon’s test was used to check the statistical significance of the difference between mean values. Results were expressed as mean±SEM.

Drugs

Phenoxybenzamine HCl (Dibenzyl ine, Smith Kline and French, Welwyn Garden City, UK), propranolol HCl (Inderal, ICI, Milan, Italy), (−)-norepinephrine bitartrate (Levophed, Winthrop-Breon, New York), and bretylium tosylate (Bretylate, Wellcome, London) were obtained from commercially available sources and diluted in fresh solutions to the desired concentrations by adding normal saline. NE-containing syringes were wrapped in aluminum foil to avoid deterioration due to light exposure.

Results

Irreversible α-Blockade by Phenoxybenzamine

In basal conditions, LBNP and exogenous NE decreased FBF by 39.8±10% and 31±7.8%, respectively. During PBZ administration, FBF increased by 134.5±24% (p<0.001) without modifications of systemic blood pressure, heart rate, and contralateral FBF. After α-adrenoceptor blockade, exogenous NE lost its vasoconstrictor effect while LBNP still caused a measurable decrement in FBF in each patient. On average, FBF decreased by 14.5±7.5%
Neurotransmission Blockade by Bretylium

Bretylium increased FBF by 144.8±21.6% (p<0.001) without changes in systemic blood pressure and heart rate. It abolished the effect of LBNP on FBF in the treated but not in the contralateral limb. Bretylium did not affect local responses to NE (control: −35.4±6.1% vs. bretylium: −40.8±7.1%) (Figure 3).

Discussion

The main finding of our study is the persistence, after α-adrenoceptor blockade, of a residual vascular response to LBNP, a stimulus whose endogenous sympathetic nature was further proved by our bretylium experiments. Considering that PBZ has some preferential α₁-antagonistic properties,¹¹,¹⁹ a contribution of postsynaptic α₂-adrenoceptors to this persistent response may be conceived, but we interpret our data to be highly consistent with the presence of sympathetic vasoconstriction not mediated by α₁-adrenoceptors⁵ even in humans. In fact, the high degree of antagonism (Figure 1) against exogenous NE, a mixed α₁- and α₂-agonist,¹⁹,²¹ suggests that complete or near-complete α-adrenoceptor blockade of either receptor subtype by the drug was achieved in our patients. Furthermore, endogenous sympathetic stimuli (such as those triggered by the LBNP maneuver) seem to interact preferentially with α₁-adrenoceptors in the human forearm.²¹ Thus, it is unlikely that results exactly similar to ours could be expected from a spared α₂-mediated vasoconstriction by PBZ. The use of irreversible antagonists, presently not available, even less selective than PBZ may clarify the problem. Furthermore, exogenous NE was delivered from the intraluminal side and probably acted on extrajunctional α₁-adrenoceptors,²²,²³ possibly more easily reached by PBZ. Therefore, we cannot exclude an only partial blockade of intrasynaptic, periadventitial α₁-adrenoceptors, that is, those preferentially activated during LBNP. However, we tend to discount this possibility because bretylium abolished LBNP-mediated vasoconstriction, indicating that effective intrasynaptic drug concentrations could indeed be achieved from the intraluminal side. Furthermore, a greater potency of PBZ against neural than against bloodborne adrenergic stimuli has been reported.²⁴ The next factor needing consideration in interpreting our results is the loss
of the presynaptic feedback control of NE release\textsuperscript{25} by \( \alpha \)-adrenoceptor blockade by PBZ. As a consequence, the amount of neurotransmitter released per unit of stimulus might have increased\textsuperscript{26} beyond either the levels attained during basal conditions or those achieved during exogenous administration. Because we could not estimate the extent of that change in endogenous NE to adjust the level of our standard exogenous infusion, an overestimation of the effect of PBZ might have resulted. This possibility might be verified by measuring local arteriovenous differences in plasma NE. However, no further changes in the vasoconstriction after \( \alpha \)-blockade could be produced in the present study by doubling PBZ concentrations, which suggests that we were probably close to or at a plateau of antagonism. It was tempting to use even higher infusion rates of PBZ, but ethical reasons prohibited this intervention.

Thus, \( \alpha \)-blockade through PBZ in human forearm arterioles blocked the effect of \( \alpha \)-adrenoceptor stimulation by exogenous NE (in the presence of propranolol) but could not abolish the vasoconstriction to endogenous sympathetic activation by LBNP. Because similar results were obtained by an even higher concentration of antagonist, sympathetic vasoconstriction not mediated by \( \alpha \)-receptors may indeed function even in humans. Our data cannot provide definite answers about the nature of this tentatively identified mechanism, but either adrenoceptors unrelated to the \( \alpha \) type, for example, the so-called “gamma receptors”\textsuperscript{7,5} or cotransmitters such as ATP, neuropeptide Y, and others might be implicated.\textsuperscript{6} In this regard, the importance of ATP in the constriction response to electrical stimulation of isolated resistance-sized arterioles was recently documented.\textsuperscript{27} Similarly, vasoconstrictor properties of neuropeptide Y have been shown in vitro\textsuperscript{28} and in animal\textsuperscript{29} studies, but a relevant role of this substance under physiologic conditions needs confirmation. Questions may also arise about the involvement in the vascular response after \( \alpha \)-blockade during LBNP of humoral vasoconstrictor factors such as angiotensin II or antidiuretic hormone. However, either no changes\textsuperscript{30} or delayed increments\textsuperscript{31} in plasma renin activity were previously reported in humans, whereas local angiotensin conversion in human forearm vessels has been ruled out.\textsuperscript{32} On the other hand, the relative insensitivity of antidiuretic hormone to volume stimuli in humans\textsuperscript{33} and the use of a short-term, nonhypotensive unloading procedure such as ours suggest no role for that hormone, whose vasoconstrictor action at physiologic concentrations was not confirmed by recent data in humans.\textsuperscript{34} Future studies are needed to further delineate the precise mechanisms of vasoconstriction not mediated by \( \alpha \)-adrenoceptors and the physiologic and physiopathologic importance of such vasoconstriction.

Acknowledgments

Art work from M. Rocchi and F. Conti is acknowledged.

References


**KEY WORDS** • sympathetic activity • α-adrenergic receptor agonists • pressure • norepinephrine • phenoxybenzamine • bretylium
Persistence of sympathetic-mediated forearm vasoconstriction after alpha-blockade in hypertensive patients.
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_Circulation_. 1989;80:485-490
doi: 10.1161/01.CIR.80.3.485

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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