Endogenous Circulating Sympatholytic Factor in Orthostatic Intolerance

Robert E. Shapiro, Bradford Winters, Mariesa Hales, Townsend Barnett, Debra A. Schwinn, Nick Flavahan, Dan E. Berkowitz

Abstract—Sympathotonic orthostatic hypotension (SOH) is an idiopathic syndrome characterized by tachycardia, hypotension, elevated plasma norepinephrine, and symptoms of orthostatic intolerance provoked by assumption of an upright posture. We studied a woman with severe progressive SOH with blood pressure unresponsive to the pressor effects of $\alpha_1$-adrenergic receptor (AR) agonists. We tested the hypothesis that a circulating factor in this patient interferes with vascular adrenergic neurotransmission. Preincubation of porcine pulmonary artery vessel rings with patient plasma produced a dose-dependent inhibition of vasoconstriction to phenylephrine in vitro, abolished vasoconstriction to direct electrical stimulation, and had no effect on nonadrenergic vasoconstrictive stimuli (endothelin-1), PGF-2$\alpha$ (or KCl). Preincubation of vessels with control plasma was devoid of these effects. SOH plasma inhibited the binding of an $\alpha_1$-selective antagonist radioligand ([125$I$]HEAT) to membrane fractions derived from porcine pulmonary artery vessel rings, rat liver, and cell lines selectively overexpressing human ARs of the $\alpha_{1A}$ subtype but not other AR subtypes ($\alpha_{1A}$ and $\alpha_{1D}$). We conclude that a factor in SOH plasma can selectively and irreversibly inhibit adrenergic ligand binding to $\alpha_{1B}$ ARs. We propose that this factor contributes to a novel pathogenesis for SOH in this patient. This patient’s syndrome represents a new disease entity, and her plasma may provide a unique tool for probing the selective functions of $\alpha_1$-ARs. (Hypertension. 2000;36:553-560.)

Key Words: receptors, adrenergic, alpha $\bullet$ hypotension $\bullet$ norepinephrine $\bullet$ baroreceptors $\bullet$ vascular diseases

One of the primary functions of the human sympathetic nervous system is maintenance of blood pressure with changes in posture, a capacity known as orthostatic tolerance. When humans assume an upright posture, gravity prompts a rapid redistribution of blood to dependent regions.$^1$ This effect must be quickly countered by physiological mechanisms to maintain venous return to the heart and adequate perfusion of the brain. Decreased venous return stimulates baroreceptors, resulting in an increase in sympathetic neural activity, stimulation of adrenergic receptors (ARs) on vascular smooth muscle by the neurotransmitter norepinephrine (NE), systemic arterial vasoconstriction, and splanchnic capacitance venoconstriction.$^2$ The physiological consequence is restoration of venous return and maintenance of blood pressure.

The $\alpha$-ARs, specifically $\alpha_1$-ARs, are known to transduce baroreceptor-mediated sympathetic traffic in capacitance and resistance vessels.$^3$ However, despite extensive investigations, details of these mechanisms have been only partly elucidated. Separate genes encoding 6 distinct $\alpha$-AR subtypes ($3 \alpha_1$-ARs and 3 $\alpha_2$-ARs) have been identified and cloned.$^4,5$ Molecular and pharmacological studies suggest significant differences in the distribution, signal transduction, and potential functionality between the $\alpha_1$-AR subtypes ($\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$).$^6,7$ Functional studies with subtype-selective antagonists$^6,8$ and differential mRNA studies$^9,10$ have helped to clarify that different $\alpha_1$-AR subtypes subserve contractile function in different vessel beds.

Selective abnormalities of the sympathetic efferent limb of the baroreflex are may occur and can result in impairments of these postural cardiovascular defenses leading to clinical dysautonomias.$^2$ In rare individuals, failure to secrete NE in response to hypotensive stimuli may result in orthostatic hypotension leading to syncope, often without compensatory cardiovascular acceleration. More commonly, abnormalities of these postural mechanisms result in clinical conditions often referred to as the “orthostatic intolerance” disorders. In these conditions, the assumption of an upright posture results in tachycardia with variable hypotension, normal or exaggerated elevations in plasma NE, and a constellation of associated symptoms: palpitations, dizziness, lightheadedness, blurry vision, fatigue, headache, shortness of breath, nausea, sweat-
ing, tremulousness, and so on. These symptoms and signs are usually relieved by lying down. With prolonged standing, some patients will lose consciousness as the result of cerebral hypoperfusion.

Orthostatic intolerance may result from cardiovascular “deconditioning” after prolonged bed rest or exposure to microgravity. Alternatively, it has been described in multiple and likely overlapping idiopathic syndromes including sympathetic orthostatic hypotension (SOH), postural orthostatic tachycardia syndrome, hyperadrenergic postural hypotension, hypersympathicotonic orthostatic hypotension, sym patheticotic orthostatic intolerance, vasoregulatory asthenia, and others. The nosology of orthostatic intolerance syndromes is evolving and likely reflects heterogeneous but currently unknown pathophysiology.

We studied a 50-year-old woman with a 10-year history of severe progressive orthostatic tachycardia and hypotension (SOH), which currently limits her standing time to <1 minute before syncope supervenes. Plasma NE concentrations were markedly elevated, and she demonstrated an absent hypertensive response to exogenous sympathimimetic drugs. On the other hand, the patient derived some elevation in blood pressure from the somatostatin analogue octreotide, which can selectively constrict splanchnic vessels independent of AR function. Taken together, these data suggest that this individual manifests a selective insensitivity to adrenergic vasoconstrictive agents rather than a generalized impairment in vasoconstriction of resistance and capacitance vessels. Furthermore, because this patient’s symptoms also transiently improved after plasmapheresis, we hypothesized that a sympathetic factor is present in this patient’s plasma that can antagonize the function of vascular α1-ARs, resulting in vascular dysregulation and the observed orthostatic intolerance.

To test this hypothesis, we examined the ability of plasma from this patient to inhibit α1-adrenergic–mediated vasoconstriction in vitro as well as its ability to inhibit binding of α1-AR ligands to their receptors. We report evidence of an endogenous macromolecule in this patient’s plasma that can (1) inhibit contraction of porcine pulmonary artery (bioassay tissue) selectively to α1-AR stimuli and (2) inhibit binding of adrenergic ligands selectively to α1b but not other α1-ARs. We propose that this patient has a novel disease process in which an endogenous discrete plasma macromolecule contributes to clinically manifest autonomic dysregulation.

Methods

Clinical History

A 50-year-old white woman was examined at 40 years of age with a long-standing history of intermittent Raynaud’s phenomenon and the subacute onset of orthostatic tachycardia with hypotension. During the 15 years before her illness, she had been a nationally ranked endurance runner, totaling >10 000 miles. Over the subsequent 10 years of illness, she had multiple chronic/progressive difficulties, including blurry vision; arthralgias without arthritis; burning dysesthesias of the feet; thermoregulatory instability with moderate hypothermia alternating with low-grade fevers, reflexive hypothermia, and spontaneous night sweats; moderate urinary retention; and fluctuating symptoms of gastroparesis, dysphagia, abdominal pain, constipation, and diarrhea. Physical examination was notable for a supine heart rate of ~55 bpm, orthostatic sinus tachycardia (>150 bpm) with hypotension rapidly progressing to syncope, bilateral tonic mydriasis, ptosis, weakness of the intrinsic muscles of the feet, pes cavus, hammer toes, and preserved deep tendon reflexes. Extensive laboratory evaluations were normal: nerve conduction studies, electromyogram, echocardiogram, Holter cardiac monitor, ECG, sinus arrhythmia to deep breathing, Valsalva ratio, routine cerebrospinal fluid studies, Schirmer’s test, urine and serum protein electrophoreses, urine heavy metal studies, total plasma volume (after prolonged fludrocortisone treatment), serum rapid plasma reagin, serum antinuclear antibodies, and serum antinicotinic acetylcholine receptor antibodies. She had a chronic iron-deficiency anemia. Histological examinations of a lumbar sympathetic ganglion, genitofemoral nerve, and lip biopsies were normal. Skin biopsies showed diminished numbers of small superficial nerve fibers in the distal extremities. Endogenous plasma NE concentrations were elevated whether supine (889 pg/mL; normal range 110 to 410 pg/mL) or standing (1113 pg/mL; normal range 120 to 700 pg/mL). Intravenous bolus infusions of phenylephrine (to 500 μg) produced no appreciable change in the supine blood pressure or heart rate (Figure 1), whereas a 200-μg bolus in control patients would be expected to raise mean arterial pressure ~30 mm Hg and produce a marked reflexive bradycardia. Treatment with oral α1-adrenergic agonists (eg, midodrine), fludrocortisone, propranolol, indomethacin, and immunosuppressant agents (corticosteroids, intravenous immunoglobulins, cyclosporine A) all failed to relieve symptoms or raise blood pressure, whereas subcutaneous octreotide or plasmapheresis could provide some transient symptomatic relief of orthostatic intolerance and hypotension. Of note is that the patient was able to maintain the upright posture for more than a few minutes when submerged in a swimming pool.

Collection of Patient Plasma

Aliquots of the plasma product retained from this plasma exchange (SOH plasma) were extensively dialyzed against Krebs-Ringer bicarbonate solution (KR) (3 hours, 4°C, MW cutoff of 10 000) (SOH DP) and then stored at ~80°C. For all experiments, the dialyzed plasma (DP) retained after plasmapheresis of a patient with chronic inflammatory demyelinating polyneuropathy (CIDP), an autoimmune disease of peripheral nerves without clinical autonomic dysfunction, was used as a control (CIDP DP). The study protocol was approved by the Johns Hopkins University School of Medicine Joint Committee on Clinical Investigations (Institutional Review Board), and all participating individuals gave informed consent. Dialyzed plasma derived from plasma exchange of several other patients was also used as control in some experimental protocols.
**Functional Studies**

SOH DP and CIDP DP (and other control plasma) were compared in their ability to block vessel contraction in vitro. Proximal pulmonary arteries (PA) were isolated from pig lungs (intralobar, generations 1 and 8)^23^ for use as the bioassay tissue according to protocols approved by the Johns Hopkins Animal Care and Use Committee. Arteries were cleaned of loose connective tissue and cut into rings 4 to 5 mm long. PA rings were then immersed in cold modified KR containing various dilutions of the SOH DP, CIDP DP, or equivalent dilutions of the diastate buffer above (control solution) for 20 hours at 4°C. The rings were then washed repeatedly in KR at 4°C and suspended horizontally between 2 stainless steel stirrups in organ chambers filled with 5 mL KR (16% O2, 5% CO2, balance N2, 37°C, pH 7.4). Dose-response curves to phenylephrine, endothelin (ET)-1, and prostaglandin F (PGF)-2α were then generated in one-half log order concentrations. In addition, to determine the influence of the SOH DP factor on sympathetic neurotransmission, in vitro contractile responses evoked by sympathetic nerve stimulation (platinum electrodes surrounding the vessel; 10 Hz, 2-ms pulses, supramaximal voltage) were determined in vascular rings (without endothelium) pretreated with SOH or CIDP DP.

**Membrane Preparation and Receptor Binding**

PA and rat liver membranes were prepared immediately after the animals were killed, according to protocols approved by the Johns Hopkins Animal Care and Use Committee. Tissues were homogenized in ice-cold homogenization buffer (10:1 wt/vol) (5 mmol/L Tris-HCl, 5 mmol/L EDTA, pH 7.4) containing protease inhibitors. Cell debris was removed by centrifugation (1000g, 5 minutes, 4°C). Membranes were pelleted by centrifugation (39 000g, 30 minutes, 4°C) and resuspended in assay buffer (150 mmol/L NaCl, 50 mmol/L Tris, 5 mmol/L EDTA, protease inhibitors, pH 7.4). The effect of the SOH DP on the binding of the high-affinity 1A–AR–specific antagonist [125I]HEAT (New England Nuclear) to (1) PA membranes, (2) membranes of recombinant rat-1 fibroblasts overexpressing individual human 1A–AR subtypes (α1A~2000 fmol/mg; α1A~1000 fmol/mg; α1D~400 fmol/mg),^3^ or (3) rat liver membranes (which express predominantly the α1B AR) was determined. For all experiments, membranes were incubated overnight at 4°C with a 1:10 dilution of SOH DP or CIDP DP. The membranes were then washed once, resuspended in assay buffer (150 mmol/L NaCl, 50 mmol/L Tris, 5 mmol/L EDTA, pH 7.4) containing protease inhibitors, and radioligand binding was performed as previously described.^23^ Prazosin (5×10^{-9} mol/L) was used to determine nonspecific binding.

**Photoaffinity Labeling**

To determine whether SOH plasma could inhibit photoaffinity labeling of α1A AR membranes, plasma membranes from recombinant rat-1 fibroblasts selectively overexpressing the α1A AR (70 μg total protein), preincubated with CIDP DP or SOH DP, were labeled with the photoaffinity ligand 125I(arylmethyl)azidoprozazin (NEN), an arylazide analogue of prazosin (150 μmol/L NaCl, 1 mmol/L MgCl2, 2.5 mmol/L EGTA, protease inhibitors, pH 7.4) in the dark. The samples were then photolyzed for 20 minutes at 4°C with a hand-held, long-wave ultraviolet lamp. After photolysis, 500 μL of ice-cold buffer was added to each sample. The samples were centrifuged for 5 minutes and pellets resuspended in Laemmli buffer and allowed to solubilize before resolution by SDS–polyacrylamide gel electrophoresis (PAGE). SDS-PAGE gels were dried and autoradiography was performed by exposure of the gel to Kodak XAR-5 film and/or analysis by PhosphorImager (Molecular Dynamics).

**Data Analysis**

Concentration-response curves were fitted to a logistic equation by means of the software PRIZM (GraphPad), and EC50 and Bmax were determined. Binding data were analyzed by nonlinear regression curve fitting (PRIZM), and Kd and Bmax, and Kd (for competition curves) were determined. Student's t tests for group comparisons between the parameters for SOH and control plasmas were performed. Results were considered significant at a value of P<0.05.

**Results**

SOH DP at dilutions of 1:4 markedly reduced maximal contraction of PA to the selective α1-adrenergic agonist phenylephrine and significantly shifted the curve to the right (Figure 2b), whereas CIDP did not (log EC50 6.37±0.04 versus 6.40±0.05; Emax 109±19% versus 110±2.3%, n=7, NS) (Figure 2a). The inhibitory effect of SOH DP was insurmountable and was characterized by a reduced maximal response (Emax 109±6.6% versus 81±1.6%; n=7, P<0.05) as well as an increase in the EC50 (log EC50 6.60±0.02 versus 5.85±0.04; n=7, P<0.05). DP in concentrations up to 1:4 from SOH or CIDP patients had no effect on potency or maximal vasoconstrictor response of PA rings to ET-1 (Emax 156±18% versus 151±9%, log EC50 7.76±0.12 versus 8.15±0.08; n=4, NS) (Figure 2c), PGF-2α, or to depolarization-induced contractions to KCl (10 to 60 mmol/L) (data not shown). Not only did SOH DP attenuate the response to phenylephrine but it abolished the response to sympathetic nerve stimulation (Figure 2d). These physiological studies suggest that dialyzed plasma from a patient with SOH might selectively inhibit α1B-AR–mediated vasoconstriction in vitro.

SOH DP inhibited radioligand binding to PA membranes by 56% (P<0.05, n=3) (Figure 3a). Radioligand binding performed on membranes from rat-1 fibroblasts expressing the different cloned human α1A–AR subtypes demonstrated a 60% reduction (P<0.05, n=4) in binding to the α1A ARs, with no significant change in number of available ligand-binding sites in membranes expressing the α1A AR, α1D AR, (Figure 3b), or αA2A–AR subtypes (data not shown). These data suggest that factor(s) in SOH plasma specifically inhibit ligand binding to the α1A AR subtype.

Saturation-binding isoforms performed and Scatchard plots constructed demonstrated no significant difference in Kd values (48±15 pmol/L [SOH] and 57±9.0 pmol/L [CIDP]), whereas SOH DP caused a 2.4-fold decrease in the Bmax (410 fmol/mg [SOH DP] versus 960 fmol/mg [CIDP]) (Figure 3c). These data confirm the reduction in receptor number measured with single-point saturation binding and suggest that the affinity of the remaining receptors is unchanged on factor binding. We observed similar results for rat liver membranes, which are known to express exclusively α1B ARs, with no significant change in number of available ligand-binding sites in membranes expressing the α1A AR, α1D AR, (Figure 3b), or αA2A–AR subtypes (data not shown). These data suggest that factor(s) in SOH plasma specifically inhibit ligand binding to the α1B AR subtype.
pK_i = −10.5 ± 0.29 (Figure 4, b and c), consistent with published values of pK_i for prazosin at the α_{1B} AR.  

**Discussion**

We have identified a patient with a unique autonomic disorder. This patient has profound orthostatic hypotension/tachycardia, symptoms of orthostatic intolerance, and exaggerated elevations of plasma NE consistent with SOH. Additionally, she has an insensitivity to the vasomotor effects of sympathomimetic drugs and numerous clinical signs and symptoms suggestive of widespread autonomic dysfunction and small-fiber neuropathy (see Clinical History, above). While the cause of this patient’s syndrome is unknown, we have detected the presence of a macromolecular (MW >10,000 kDa) factor in her plasma that can (1) selectively inhibit contraction of porcine proximal pulmonary arteries to α_{1}-AR agonists and sympathetic nerve stimuli in vitro and (2) selectively inhibit binding of α_{1}-AR antagonist radioligands to the α_{1B} AR subtype. We propose that this circulating substance contributes to the pathogenesis of this patient’s disorder.

The pathophysiology of orthostatic intolerance disorders, including SOH, are poorly understood, and responses of individuals to therapies are inconsistent. Similar vasomotor changes have been observed after the deconditioning of prolonged bed rest or exposure to microgravity during space-flight. These abnormalities are often accompanied by reduced plasma renin levels. It has been speculated that SOH may be caused by an “abnormal venodilator,”31 as in hyperbradykininism, or result from “impaired effector organ responses” to sympathetic activity. Dysfunctions of adrenoreceptors have been proposed, but data in support of these hypotheses are generally scant. One group, however, has reported evidence of exaggerated responses of α_{2}-ARs in association with SOH and mitral valve prolapse. These and other observations (eg, increased plasma catecholamines) have led to consideration of a “hyperadrenergic state” in some patients with orthostatic intolerance. The nature of such a state is unclear. Patients with orthostatic intolerance often benefit therapeutically more from plasma volume expansion (eg, salt/volume loading) and α_{1}-sympathomimetic drugs (eg, midodrine) than from the α_{2}-AR agonist clonidine, which would antagonize central sympathetic outflow. These data argue against the presence of a central hyperadrenergic state but rather that peripheral sympathetic mechanisms fail to redistribute blood volume in the face of orthostatic challenge because of hypovolemia or loss of vascular tone. Cumulatively, these various observations appear to reflect a heterogeneity of causes for SOH and orthostatic intolerance.

A currently favored hypothesis attributes the development of orthostatic intolerance in many patients to a “partial” autonomic neuropathy. According to this hypothesis, a subset of peripheral sympathetic nerve fibers degenerate in a length-dependent fashion as the result of an as-yet unidentified pathogenic process. Evidence presented in favor of partial autonomic neuropathies in such patients includes frequent observations of small-fiber neuropathies, selective pooling or idiopathic reductions in plasma volume and/or red cell volume. These abnormalities are often accompanied by reduced plasma renin levels. It has been speculated that SOH may be caused by an “abnormal venodilator,” as in hyperbradykininism, or result from “impaired effector organ responses” to sympathetic activity. Dysfunctions of adrenoreceptors have been proposed, but data in support of these hypotheses are generally scant. One group, however, has reported evidence of exaggerated responses of α_{2}-ARs in association with SOH and mitral valve prolapse. These and other observations (eg, increased plasma catecholamines) have led to consideration of a “hyperadrenergic state” in some patients with orthostatic intolerance. The nature of such a state is unclear. Patients with orthostatic intolerance often benefit therapeutically more from plasma volume expansion (eg, salt/volume loading) and α_{1}-sympathomimetic drugs (eg, midodrine) than from the α_{2}-AR agonist clonidine, which would antagonize central sympathetic outflow. These data argue against the presence of a central hyperadrenergic state but rather that peripheral sympathetic mechanisms fail to redistribute blood volume in the face of orthostatic challenge because of hypovolemia or loss of vascular tone. Cumulatively, these various observations appear to reflect a heterogeneity of causes for SOH and orthostatic intolerance.

A currently favored hypothesis attributes the development of orthostatic intolerance in many patients to a “partial” autonomic neuropathy. According to this hypothesis, a subset of peripheral sympathetic nerve fibers degenerate in a length-dependent fashion as the result of an as-yet unidentified pathogenic process. Evidence presented in favor of partial autonomic neuropathies in such patients includes frequent observations of small-fiber neuropathies, selective pooling or idiopathic reductions in plasma volume and/or red cell volume. These abnormalities are often accompanied by reduced plasma renin levels. It has been speculated that SOH may be caused by an “abnormal venodilator,” as in hyperbradykininism, or result from “impaired effector organ responses” to sympathetic activity. Dysfunctions of adrenoreceptors have been proposed, but data in support of these hypotheses are generally scant. One group, however, has reported evidence of exaggerated responses of α_{2}-ARs in association with SOH and mitral valve prolapse. These and other observations (eg, increased plasma catecholamines) have led to consideration of a “hyperadrenergic state” in some patients with orthostatic intolerance. The nature of such a state is unclear. Patients with orthostatic intolerance often benefit therapeutically more from plasma volume expansion (eg, salt/volume loading) and α_{1}-sympathomimetic drugs (eg, midodrine) than from the α_{2}-AR agonist clonidine, which would antagonize central sympathetic outflow. These data argue against the presence of a central hyperadrenergic state but rather that peripheral sympathetic mechanisms fail to redistribute blood volume in the face of orthostatic challenge because of hypovolemia or loss of vascular tone. Cumulatively, these various observations appear to reflect a heterogeneity of causes for SOH and orthostatic intolerance.
abnormalities of venoconstriction, and reduced plasma renin levels. According to the hypothesis, the latter 2 observations may result from the selective loss of renal and venular sympathetic innervation.

Our data suggest that an alternate hypothesis may account for these observations. We propose that the observed abnormalities in venous tone and the reduced renin levels leading to hypovolemia may be due to selective reductions in the responsiveness of renal and venous sympathetic end-organ.

Such hyporesponsiveness could be caused by a circulating sympatholytic factor (e.g., α1B AR antagonists) comparable to what we have detected in our patient or by alternate mecha-

Figure 3. a, [125I]HEAT binding demonstrating 56% inhibition of radioligand binding to membranes prepared from porcine PA preincubated with SOH plasma. b, SOH plasma significantly inhibits radioligand binding to membranes prepared from cells transfected with human recombinant α1B AR but not α1A AR or α1D AR. c, Saturation isotherms of membranes prepared from rat-1 fibroblasts transfected with human recombinant α1B AR, and d, membranes prepared from rat liver (which express exclusively α1B AR) and their corresponding Scatchard plots (e and f) demonstrate significant decrease in B_max but no significant change in the K_d, suggesting that SOH plasma factor acts as noncompetitive inhibitor of ligand binding.
Figure 4. a, [125I]azidoprazosin labeling of rat-1 fibroblasts expressing human recombinant α1B AR preincubated with CIPD plasma (control) or SOH plasma (autoradiogram of SDS-PAGE gel). This confirms inhibition observed in classic binding with a second radioligand and localizes it to a band with electrophoretic mobility comparable to that predicted for α1B AR. b, Autoradiogram of typical SDS-PAGE gel and plot of curve after densitometric analysis of gel bands. pK_i for prazosin is −10.5, consistent with previously published pK_i for inhibition of prazosin binding. This confirms that α1B AR is labeled by azidoprazosin in these membranes.

Considerable data support the contention that dysfunctional α1B ARs may play a role in the pathogenesis of orthostatic intolerance. Information regarding α1B AR signaling in the vasculature has been obtained from 3 sources: (1) the use of subtype-specific pharmacological antagonists, (2) RNA expression studies, and (3) preliminary studies in an α1B AR knockout mouse. The problems associated with the use of subtype-specific antagonists is that the available agents are not completely subtype specific, making interpretation of antagonist-based experiments more difficult. The limitation with RNA and immunological expression studies is that functionality must be inferred. This is further compounded by the problem of significant species heterogeneity in subtype expression. Given these caveats, it is increasingly evident that the α1B AR is important in mediating the arterial and venoconstrictor responses to NE in the vasculature. Available studies show that α1B ARs mediate both venoconstriction and arterioconstriction in rat. The subtype distribution of α1B-ARs is well summarized in Reference 24 according to species and vessel type. For example, in a recent study that used a combination of immunological, molecular biological, and pharmacological approaches, Piascik et al demonstrated that despite widespread α1B AR immunoreactivity, functional studies suggest that the α1B AR mediates constriction only in the mesenteric resistance arteries. Leech et al demonstrated that the α1A mediates contractile responses in the cremaster skeletal muscle arterioles, whereas the α1B AR mediates contractile responses in the veins. In addition, the α1A AR is the predominant subtype expressed in rat portal vein. There are few data regarding human subtype-specific vascular function. The few studies performed to date have identified all 3 subtypes in mesenteric arteries, whereas the α1B AR is expressed in aorta. The α1B mediates constriction in the superior vesicle and obturator arteries. The expression of α1B-AR receptor subtypes and their function is lacking in human veins, particularly the microvasculature. The role of the α1B AR in vascular contractile responses in general is also consistent with studies of the α1B AR “knockout” mouse, in which a 45% reduction in the mean arterial blood pressure response to phenylephrine was observed in mice lacking α1B ARs. Furthermore, α1A-ARs expressed in human kidney are almost exclusively of the α1B subtype, an observation that may relate to the presence of reduced renin levels in some patients with orthostatic intolerance. Thus, despite the paucity of data on human expression of α1-AR subtypes and heterogeneity of subtype expression across species, there is ample evidence that the α1B AR is critical in mediating vascular contractile responses and that impairment in these responses could indeed lead to orthostatic intolerance. Because of the lack of subtype-specific functional human data, the clinical presentation of our patient currently represents the only data available that reflect the physiological consequences of the selective antagonism of α1B ARs in humans. Our data suggest that the α1B AR subtype is crucial to the mediation of adrenergic responses to orthostatic challenges in humans.

Streiten and Sculld have presented striking data indicating an augmented (“supersensitive”) vasoconstrictor response of foot veins to infused NE in 22 of 32 patients with orthostatic intolerance. These authors hypothesized that these abnormal responses reflected “probable upregulation of venous α-adrenergic receptors.” Two patients in their study, however, demonstrated subnormal constrictor responses to infused NE that were interpreted as a “malfunction at a receptor or postreceptor site in the venous contractile mechanism.” If these interpretations are correct, these provocative data indicate that sympathetic end-organ hyporesponsiveness is likely a relatively rare cause for orthostatic intolerance.
Despite the complex way in which the $\alpha_1$-ARs are regulated, it is clear that chronic increased agonist exposure results in the downregulation (decreased expression) and uncoupling of $\alpha_1$-ARs from their signal transduction mechanisms. The functional consequence of this could be a further exacerbation of the attenuated contractile responses to NE release after an orthostatic challenge in this patient.

The characterization of a macromolecular sympatholytic factor in our patient raises the possibility that endogenous modulators of $\alpha_1$-ARs may be dysregulated in this and other disorders. Endogenous antibodies to ARs have been demonstrated, some with pathological consequences, in malignant hypertension, congenital heart block, and asthma. Although the pharmacological characteristics of the plasma macromolecules detected in our patient are unique (no known agent has similar antagonist characteristics of the plasma macromolecules detected in our patient are unique), the biochemistry of the $\alpha_1$-ARs is unknown. Preliminary data suggest that the factor is resistant to proteolysis (trypsin or Pronase) and is resistant to the denaturing effect of boiling (data not shown). These findings make it improbable that the sympatholytic factor is a large globular protein such as an immunoglobulin. Identification of the physicochemical nature of this endogenous factor could lead to the identification of its sites of production, genetics, site of action, and possible normal physiological functions. These studies may lead to insights into the causes of more common diseases of vascular dysregulation such as hypertension and orthostatic hypotension or physiological “deconditioning” responses seen after bed rest or spaceflight.

Our physiological data support the hypothesis that $\alpha_1B$ ARs are the primary mediators of sympathetic neurotransmission in porcine pulmonary artery: SOH-dialyzed plasma attenuated phenylephrine-induced vasoconstriction but completely abolished endogenous neuronal sympathetic neurotransmission in vitro. These observations may reflect a differential distribution of $\alpha_1$-AR subtypes within individual blood vessels. That is, $\alpha_1B$ ARs may be preferentially localized to subserve sympathetically mediated vasoconstriction (ie, near sympathetic neuroeffector junctions). Expression of extrajunctional receptors of a different subtype may account for the residual response to phenylephrine. If $\alpha_1B$ ARs (with relatively low affinity for NE) are primarily junctional receptors in PA, then they may be positioned to respond to a broad physiological range of NE (ie, high concentrations of NE at the neuroeffector junctions as well as lower concentrations of circulating NE). On the other hand, extrajunctional receptors with higher affinities (perhaps $\alpha_1A$ ARs) may respond only to circulating epinephrine or NE. This hypothesis remains to be tested.

Which properties of the $\alpha_1B$ ARs render them selectively sensitive to the sympatholytic factor in SOH plasma? $\alpha_1$-ARs are members of the large family of $G$ protein–coupled plasma membrane receptors. These receptors share a serpentine homologous tertiary structure that is modified with oligosaccharides close to the amino terminus. The 3 $\alpha_1$-AR subtypes differ considerably with respect to site and degree of glycosylation as well as having regionally divergent amino acid sequences. The noncompetitive nature of the radioligand-binding antagonism of the SOH plasma factor suggests that it inhibits binding to $\alpha_1B$ ARs at a site distinct from the ligand binding site and possibly based on steric factors. Studies with chimeric $\alpha_1$-AR (ie, $\alpha_1A/\alpha_1B$) could help clarify the sites of action of the SOH plasma factor.

The detection of a selective sympatholytic factor against $\alpha_1B$ ARs in our patient’s plasma represents a new paradigm for considering the pathogenesis of orthostatic intolerance. The prevalence of sympatholytic factors in such patients is currently unknown and awaits further investigation.

Acknowledgments

This work was supported in part by a grant from the Berman Foundation (Dr Shapiro), Richard Ross Clinician Scientist award, Johns Hopkins University School of Medicine (Dr Berkowitz), National Space Biomedical Research Institute Grant NCC 9-58-0 (Dr Berkowitz), and National Institutes of Health grant AG00745 (Dr Schwinn). We would like to thank Cheryl Dwyer for excellent secretarial support. The authors gratefully acknowledge the cooperation and support of the patient reported herein.

References


Endogenous Circulating Sympatholytic Factor in Orthostatic Intolerance
Robert E. Shapiro, Bradford Winters, Mariesa Hales, Townsend Barnett, Debra A. Schwinn, Nick Flavahan and Dan E. Berkowitz

Hypertension. 2000;36:553-560
doi: 10.1161/01.HYP.36.4.553
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 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/36/4/553

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/