MATERIALS and METHODS SUPPLEMENT

Subjects

The diagnosis of VVS was primarily based on clinical history. We took a detailed medical history and performed physical examination, electrocardiography, echocardiography, and prolonged monitoring as needed to exclude cardiac and other causes of fainting. Prolonged standing produced fainting in all patients. Three VVS patients also fainted in response to noxious stimuli. Prodromal signs and symptoms included lightheadedness, pallor, nausea with abdominal discomfort, warmth, diaphoresis, visual scotomata or loss of vision. Consciousness was restored within 30s once supine. Most patients experienced post-faint fatigue and headache. Exclusion criteria for participation in the study included any systemic or infectious disease, other forms of orthostatic intolerance, competitive athletic training, recent long-term bed rest, use of nicotine, or pregnancy. Cardiovascular disease was specifically excluded. All medications were stopped for at least 2 weeks prior to the study. No subjects were taking vasoactive or neurally-active drugs.

VVS and healthy volunteer subjects previously had a screening upright tilt table test to 70^{o} in which signs and symptoms of real-world syncope were confirmed in VVS patients and absent in controls during a 10-min upright tilt without pharmacologic provocation. All subjects refrained from caffeine and xanthine-containing products for at least 72 hours prior to testing and fasted for at least 4 hours prior to testing.

Instrumentation

The lower body (legs and hips) of supine subjects were placed in an airtight chamber hermetically sealed at the iliac crest with a rubber diaphragm. A left antecubital vein catheter was placed for infusion of L-NMMA or for saline+PE. Beat-to-beat blood pressure was measured by Finometer photoplethysmograph (FMS, Amsterdam) on the right forefinger or middle finger calibrated to the brachial artery. The Finometer uses the Modelflow algorithm to estimate beat-to-beat CO by pulse-wave analysis. Before experiments began, ModelFlow CO was calibrated against an Innocor inert gas rebreathing CO (Innovision, Denmark). Respiratory plethysmography (Respitrace, NIMS Scientific) and capnography (Smith Medical PM) measured changes in respiration and end tidal carbon dioxide ($ETCO_2$). An electrocardiograph measured HR from the beat-to-beat cardiac electrical intervals. Signals were acquired at 200 samples/s, multiplexed, and A/D converted using custom software. Wire connections were externalized through airtight ports. Suction was provided by a vacuum pump controlled with a variable transformer calibrated against a manometer. The device can accurately apply pressures between 0 (atmospheric) and -60mmHg within a few seconds.

LBNP

During -60mmHg LBNP we made repeated measurements every 5 min; we denoted the first 5 min as -60(1), the second as -60(2) and so on. Stopping criteria for presyncope in all subjects were defined as a decrease in systolic BP to 80 mmHg; a decrease in systolic BP to 90 mmHg associated with lightheadedness, nausea or sweating; or progressive symptoms of presyncope accompanied by a request from the subject to

stop the test. LBNP was the preferred orthostatic stress as it allowed subjects to remain supine which facilitated the complex instrumentation performed. Statistics were computed by including post-loading data (L-NMMA or Saline+PE) and the LBNP responses.

L-NMMA infusion

VVS patients and control subjects received the non-isoform specific NOS inhibitor L-NMMA delivered as a 500μg/kg/min intravenous loading dose for 15 min, followed by a 50μg/kg/min maintenance infusion. Maintenance L-NMMA continued throughout all subsequent measurements. L-NMMA is the only parenteral experimental NOS inhibitor available for human use in the USA (Bachem, Switzerland; FDA IND exemption #76,314, J. Stewart).

Data Analysis

Not all subjects were able to reach all levels of LBNP before achieving stopping criteria, especially during Saline+PE. However, all patients were able to reach -15, -30, and -45 mmHg of LBNP; these pressures were used for statistical comparison of VVS and healthy control subjects during Saline+PE. Some VVS patients reached -60 mmHg during Saline+PE. If so, we showed these data recorded 1 min before stopping in our graphics but did not use these data for statistical comparison. Similarly, not all subjects were able to reach all levels of LBNP during L-NMMA infusion before achieving stopping criteria. However, all patients were able to reach -15, -30, -45, and 5 min of -60 mmHg (-60(1)), these pressures were used for statistical comparison of VVS and healthy control subjects during L-NMMA.

The primary outcome variables were BP, HR, CO, TPR, and time to stopping LBNP to measure the orthostatic intolerance threshold; secondary outcome variables were changes in splanchnic, pelvic and calf blood flows measured by impedance plethysmography, corresponding regional blood volume changes and related regional resistances calculated as MAP/flow [8]. Forearm blood flow was also measured by venous occlusion plethysmography every 5 min [21]. Thoracic (central) blood flow was measured as the CO.