Endogenous substance P modulates human cardiovascular regulation at rest and during orthostatic load

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Dzurik MV, Diedrich A, Black B, Paranjape SY, Raj SR, Byrne DW, Robertson D. Endogenous substance P modulates human cardiovascular regulation at rest and during orthostatic load. J Appl Physiol 102: 2092–2097, 2007. First published January 25, 2007; doi:10.1152/japplphysiol.00969.2006.—Substance P (SP) is a peptide neurotransmitter identified in many central and peripheral neural pathways. Its precise role in human physiology has been difficult to elucidate. We used the selective neurokinin 1 (NK1) antagonist aprepitant as a pharmacological probe to determine the role of endogenous SP in human cardiovascular regulation. We performed a randomized, double-blind, placebo-controlled, crossover trial in healthy subjects. Blockade of endogenous NK1 receptors reduced resting muscle sympathetic activity 38% (P = 0.002), reduced systemic vascular resistance by 25% (P = 0.021), and increased cardiac index by 47% (P = 0.006). This constellation of changes did not, however, alter either blood pressure or heart rate in the supine position. NK1 antagonism also raised orthostatic heart rate change by 38% (P = 0.023), although during the incremental postural adjustment on the tilt table neither heart rate nor blood pressure was altered significantly. Despite a mildly attenuated vagal baroreflex with SP blockade, the depressor and pressor responses to nitroprusside and phenylephrine did not differ compared with placebo, suggesting other compensatory mechanisms. NK1 blockade manifests as a decrease in muscle sympathetic nerve activity and systemic vascular resistance. Our study suggests SP exerts a tonic enhancement of sympathetic outflow to some cardiovascular structures via its modulation of the NK1 receptor. Most likely, this ubiquitous neurotransmitter exerts effects at multiple sites that, in the aggregate, are relatively well compensated under many circumstances but may emerge with perturbations. This study is consistent with a role for SP afferents in supporting peripheral vascular resistance.

Neurokinin 1; tachykinin; blood pressure; syncope; hypertension

Substance P (SP) is a decapeptide widely distributed in the central, peripheral, and enteric nervous system that acts as a neurotransmitter with many putative functions (7, 10, 22, 24). It is a member of the tachykinin family of peptides, which interact with tachykinin receptors designated as neurokinin-1 (NK1), NK2, and NK3 (4). The effects of SP are mediated primarily by the NK1 receptor. In some animal models, blockade of NK1 receptors attenuates the baroreflex. Despite a wealth of animal data showing that SP is involved in nociceptive and cardiovascular mechanisms (2, 3, 10, 15–17, 19, 20) and in the nucleus tractus solitarii (2, 5, 20, 22), there is nevertheless little current human data implicating a role for SP in the modulation of cardiovascular function. It has even been suggested that SP may not contribute to basal vascular tone in human subjects (18). There remains a disconnect between the wide distribution of neuronal SP in the viscera, in afferent neurons in various distributions, and in the central nervous system on the one hand, and the paucity of clear evidence implicating this neurotransmitter in cardiovascular regulation in human subjects. A major reason for the limited human data on the role of SP has been a lack of potent and selective antagonists of neurokinin receptors available for human subjects.

Aprepitant is a highly selective inhibitor of the NK1 receptor (14) that is FDA approved as an adjunct for antiemetic therapy in patients receiving chemotherapy (6). High levels of occupancy of central NK1 receptors by this agent were documented by PET (1). Peripheral receptor antagonism at relatively low doses of NK1 blockade was also achieved (23). Availability of a potent and selective antagonist in human subjects holds the promise that we may use aprepitant as a pharmacological probe to explore the role and net effect of endogenous SP on cardiovascular function.

The objective of this study was to assess the effect of NK1 receptor blockade by aprepitant on autonomic cardiovascular control and the baroreflex.

METHODS

Study Hypothesis

Pharmacological block of endogenous NK1 receptors attenuates the cardiac baroreflex in healthy volunteers.

Study Design

A prospective, randomized, double-blind, placebo-controlled crossover study was performed on 12 healthy volunteers (11 men, 1 woman) to assess the effect of NK1 receptor block on baroreflex function, muscle sympathetic nerve activity, hemodynamics, and plasma catecholamines and their metabolites.

Subjects

Healthy study volunteers (age 22–48 yr), without a history of syncope or presyncope, were recruited from the Vanderbilt community database. Exclusion criteria included medical illness, a history of syncope, presyncope, hypertension, or smoking. Screening required a normal history and physical, electrocardiogram (ECG), complete blood count, complete metabolic panel, urinalysis, and urine preg-
nancy test (if applicable). A total of 14 subjects gave their consented for this study, and 12 completed the protocol. All subjects provided written, informed consent. The Vanderbilt Institutional Review Board approved this study.

Randomization and Blinding

The Vanderbilt Investigational Pharmacy randomly assigned study participants to either placebo or aprepitant during phase 1, with a crossover in phase 2. The placebo was created to match the study drug in size, shape, weight, and taste. The principal investigator and participants remained blinded until the completion of the study by all subjects.

Interventions and Compliance

Subjects were provided with a controlled diet 48 h before administration of the study drug. This consisted of an alcohol-free, methylxanthine-free, normal sodium (150 meq/day), and normal potassium (70 meq/day) diet. Study drug was administered between 2000 and 2200 and documented by the General Clinic Research Center nursing staff.

Subjects received an oral loading dose of 120 mg of aprepitant (Emend, Merck, & Co.) or placebo administered the evening before day 1 and a second dose of 80 mg on the second evening. Previously obtained pharmacokinetic data were provided by Merck; these suggested that peak levels occur 3–4 h after administration with equal serum concentrations on each day of testing. However, Merck provided neither drug nor financial support for any aspect of this study.

Evaluations were performed with patients in a fasting state. Day 1 of testing consisted of placement of a peripheral intravenous catheter, followed by microneurography of the peroneal nerve. Use of these methods in elucidation of autonomic cardiovascular regulation has been previously described (23). With microneurographic apparatus in position, the subjects underwent autonomic function and pharmacological testing. Subjects performed the Valsalva maneuver, static handgrip, and cold pressor tests. After a rest period with a return of hemodynamic data to baseline, continuous infusions of phenylephrine and nitroprusside were then administered in escalating doses (0.2, 0.4, 0.8, 1.6 µg·kg\(^{-1}\)·min\(^{-1}\)) every 5 min. There was a rest period of at least 10 min between infusions of nitroprusside and phenylephrine, with a return of hemodynamic parameters to baseline. Continuous recording of ECG, heart rate (HR), respiration, spihgmonomanometric blood pressure (SBP; Vital-Guard 450C, Ivy Biomedical Systems, Branford, CT), finger blood pressure (Finapress 2300, Ohmeda, Madison, WI), and muscle sympathetic nerve activity (662C-3 Nerve Traffic Analysis System, Univ. of Iowa, Iowa, IL) were recorded and digitized at a sampling rate of 500 Hz using DI-720USB and Windaq Pro+ software (DATAQ Instruments, Akron, OH). This data was then processed with user software written in PV-Wave (Visual Numerics, Houston, TX) obtained on a personal computer. End points included an increase or decrease in cuff blood pressure of >25 mmHg or achieving maximum dose.

The morning of day 2, subjects underwent a posture study that consisted of obtaining plasma catecholamines and their metabolites while supine and after standing upright for 30 min. Plasma was separated by centrifugation at −4°C, transferred to collection tubes with 40 µl/ml plasma of 6% glutathione (Sigma Scientific, St. Louis, MO), and stored at −70°C until the assay was performed. The samples were then measured by batch alumina extraction followed by high-performance liquid chromatography for separation with electrochemical detection and quantification. During the posture study, SBP was evaluated every 5 min, followed by plasma catecholamines at the end of the study. Patients then received 8 oz. of water by mouth to correct for overnight dehydration. A graded head upright tilt table test (HUT) was performed (B3530- VS Beta Plus, Berne Manufacturing, Columbia, SC) with continuous monitoring of ECG, HR, respiration, SBP, finger blood pressure, and bioelectrical body impedance (KIM4 Heinz Diefenbach, Frankfurt, Germany). The table was raised to 75 degrees in 15-degree increments every 5 min. Total duration of the protocol was 45 min. Plasma catecholamines were sampled at baseline (as previously described), during the last 2 min at each 15-degree increment, and just before return to the supine position. This completed phase 1. Subjects underwent a 1-wk washout period; then this protocol was repeated in the crossover phase with the alternate study drug.

Statistics

With no human data available, a sample size of 10 was calculated for this study to have 80% statistical power to detect a clinically significant difference in population means of 10 (e.g., 10 mmHg or beats/min) with a standard deviation in response of matched pairs of 10. This calculation was performed using the PS sample size software (Dupont), assuming a two-sided alpha level of 0.05 and based on a paired t-test.

The baroreflex sensitivity was calculated by plotting the change from baseline in the average HR (ms) from the final 2 min of drug infusion, after a steady state had been obtained, against the corresponding blood pressure. The slope of the derived linear regression formula for these points was taken to indicate vagal-mediated baroreflex sensitivity. Microneurography data was expressed as number of bursts per minute. The change in blood pressure was then plotted against the corresponding muscle sympathetic nerve activity, and the slope of the derived linear regression formula was used to evaluate the sympathetic limb of the cardiac baroreflex. Each subject’s baroreflex sensitivity was calculated individually using both the systolic and diastolic blood pressure. Cardiac index was determined from the body impedance data as previously described utilizing the Kubicke method (11). HR variability was calculated from the resting ECG. Reliable signals were obtained in nine of the volunteers. Results from phases 1 and 2 were compared using a paired t-test as the data were normally distributed. A probability of <0.05 was considered significant. Plasma catecholamine levels were compared at baseline and each phase of HUT using repeated-measures ANOVA. Hemodynamic data from the HUT were compared using repeated-measures ANOVA and displayed graphically with 95% confidence intervals. Statistical analyses were performed using SPSS for Windows software version 13.0 (SPSS, Chicago, IL). All tests were two tailed.

RESULTS

Resting Data

Randomization. Eight of 12 volunteers began phase 1 on placebo.

Hemodynamics. There was no difference in blood pressures or HRs at rest. Data obtained from body impedance recordings during baseline demonstrated a 25% decrease from 817 to 616 dyn·s/cm\(^5\) [95% confidence interval (CI) for Δ201, 39–362, P = 0.021] in the resting systemic vascular resistance during the aprepitant phase, with a 47% increase in the resting cardiac index; 4.3 to 6.3 (95% CI for Δ2, 0.8–3.2, P = 0.006) l/min·m\(^{-2}\), and a 27% increase in the stroke volume from 147 to 202 ml (95% CI for Δ55, 14–96, P = 0.015) (Fig. 1). HR variability measurements showed no significant differences in the low-frequency signal during placebo vs. aprepitant phase (2.466 to 2.266 ms\(^2\); 95% CI for Δ200, −2.120 to 2.521, P = 0.851), high frequency (846 to 666 ms\(^2\); 95% CI for Δ180, −297 to 656, P = 0.421), low frequency-to-high frequency ratio (3.3 to 3.1; 95% CI for Δ0.2, −1.3 to 1.5, P = 0.833), or the total power (6,369 to 5,187 ms\(^2\); 95% CI for Δ1,182, −4,214 to 6,579, P = 0.636).
Muscle sympathetic nerve activity. Eight of the 12 subjects had adequate peroneal nerve isolation during both phases to afford data for analysis. In these individuals, there was a 38% decrease in resting muscle sympathetic nerve activity during the aprepitant phase, from 19.6 to 12.1 bursts/min (95% CI for \( < 0.001 \), 7.5–11.2, \( P = 0.002 \)) while on the placebo phase (Fig. 2).

Orthostatic and Pharmacological Loading

Hemodynamics. Although the change in the blood pressure on standing showed no significant differences (Table 1), there was a 38% greater increase (\( P = 0.023 \)) in HR, from 13.3 to 21.4 beats/min (95% CI for \( \Delta 8.1, 1.4 \text{–} 14.8 \)), during the posture study of the aprepitant phase (Fig. 3). Interestingly, during HUT, there was no difference in the systemic hemodynamics at baseline (SBP placebo 110 ± 4 vs. SBP aprepitant 110 ± 8 mmHg; HR placebo 63 ± 6 vs. HR aprepitant 61 ± 4 beats/min) or at end tilt (SBP placebo 121 ± 10 vs. SBP aprepitant 129 ± 6 mmHg; HR placebo 81 ± 7 vs. HR aprepitant 80 ± 7 beats/min). Three of 12 subjects had positive tilt tests (presyncope and hypotension) during both phases of the study. Three of 12 subjects had a positive tilt test during the aprepitant phase and a negative test while on placebo.

Baroreflex sensitivity. The vagal component of the cardiac baroreflex was attenuated slightly but significantly with NK1 receptor blockade using the SBP for analysis (Figs. 4 and 5). The mean baroreflex sensitivity decreased from 18.2 to 15.1 ms/mmHg (95% CI for \( \Delta 3.1, 0.5 \text{–} 5.8, P = 0.022 \)). When we subsequently analyzed the baroreflex slope as a function of the diastolic blood pressure, the data were analogous but did not reach significance, with a decrease from 23.7 to 19.8 ms/mmHg (95% CI for \( \Delta 3.9, -0.8 \text{ to } 8.6, P = 0.097 \)). There was no significant decrease in the sympathetic component of the cardiac baroreflex, \( 0.99 \text{ to } 0.80 \text{ mmHg burst}^{-1} \text{min}^{-1} \) (95% CI for \( \Delta 0.19, -0.64 \text{ to } 0.28, P = 0.379 \)). There was no difference in the SBP response during pharmacological challenge (maximal ASBP with phenylephrine, placebo 19 ± 4 vs. aprepitant 23 ± 6 mmHg; nitroprusside placebo 13 ± 8 vs. aprepitant −14 ± 5 mmHg) (Fig. 6).

Autonomic function testing. There was no difference in the Valsalva ratio (\( P = 0.768 \)), blood pressure response to static hand grip (\( P = 0.236 \)), or blood pressure response to cold pressor (\( P = 0.186 \)) between aprepitant and placebo. Muscle sympathetic nerve activity activity measured during autonomic function testing demonstrated a blunting in the sympathetic response to handgrip from 23.2 to 15.7 bursts/min (95% CI for

### Table 1. Blood pressure and heart rate during supine and upright posture

<table>
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<th>Supine</th>
<th>Upright</th>
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<tr>
<td>SBP, mmHg</td>
<td>117±10</td>
<td>117±20</td>
<td>115±10</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>68±10</td>
<td>76±14</td>
<td>67±9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>60±12</td>
<td>73±11</td>
<td>58±8</td>
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<tr>
<td>( \Delta )SBP</td>
<td>0±18</td>
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<td>1±11</td>
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<tr>
<td>( \Delta )DBP</td>
<td>8±8</td>
<td>5±8</td>
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<tr>
<td>( \Delta )HR</td>
<td>13±9</td>
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Values are means ± SD, and compared between placebo and aprepitant. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.
There was no significant difference in sympathetic activity during Valsalva maneuver, \(38.0 \text{ to } 33.5\) burst/min (95% CI for \(4.5 \text{ to } 9.0\), \(P = 0.461\)), or cold pressor, \(26.0 \text{ to } 26.1\) burst/min (95% CI for \(-11.7 \text{ to } -11.4\), \(P = 0.974\)).

**Plasma catecholamines.** There was no difference in the levels of plasma norepinephrine (\(P = 0.365\)), epinephrine (\(P = 0.456\)), dopamine, dihydroxyphenylglycol, or dihydroxyphenylacetic acid at baseline or during phases of head-up tilt (Fig. 7). Likewise, in both the supine and upright phases of the posture study, plasma levels were within normal limits and were not altered by NK\(_1\) blockade.

**DISCUSSION**

NK\(_1\) receptor blockade reduces muscle sympathetic activity by 38%, reduces systemic vascular resistance by 25%, and increases cardiac index by 47%. Although bioelectric body impedance data are known to overestimate the actual cardiac output and stroke volume used to calculate this index, the crossover nature of this study enables us to expect that the differences encountered in our study are valid. This constellation of changes did not, however, alter either blood pressure or HR in the supine position.

A second new finding is that NK\(_1\) antagonism raised HR with standing by \(-38\%\), although the incremental postural adjustment on the tilt table did not significantly alter either HR or blood pressure significantly. The norepinephrine level during upright tilt was not significantly altered. Despite the small decline in baroreflex slope with SP blockade, the blood pressure responses of the patients to nitroprusside and to phenylephrine did not differ after NK\(_1\) blockade vs. placebo.

The finding of significantly altered muscle sympathetic activity in a setting of unaltered plasma norepinephrine, although not by any means unprecedented, is nevertheless unusual. It is possible that subtle differences in norepinephrine levels somehow escaped our detection in this study or, more likely, that altered release of norepinephrine was disguised by concomitant and reciprocal changes in norepinephrine metabolism or clearance. We cannot address this directly because we did not carry out norepinephrine clearance studies during this investigation. We did, however, measure plasma dihydroxyphenylglycol, dopa, dihydroxyphenylacetic acid, dopamine, and epinephrine levels in the same samples in which we measured plasma norepinephrine but did not detect significant differences in these either. A more likely scenario is that, with SP antagonism, regional differences in muscle sympathetic nerve activity change occurred, which somehow resulted in well compensated circulate nanogram norepinephrine levels.

The three neurokinin receptors manifest their actions via G-protein coupling. Eight tachykinin peptides have been iden-
Identified with relative receptor selectivity. Although SP exerts its primary effect via NK₁, other tachykinins may act as full agonist for this particular receptor. The potency depends on the G protein involved in the signal transduction. In general, SP has the strongest affinity for NK₁, followed by human hemokinin-1/H₁, human hemokinin-2/NKA/H₁, and human NKB.

For many years, there was considerable debate about the possibility that SP might be the principal neurotransmitter in the afferent side of the baroreflex (10). This focused much attention to a potential mechanism by which SP might be involved in cardiovascular regulation. The selective destruction of neurons with NK₁ receptors in the NTS eliminates most baroreflex function and underscores the presence of this receptor type on neurons crucially involved in mediation of this important function (22).

Recent studies by Williams et al. (26) support the view that the SP involved in mediating peripheral somatomotor signal input to the rostral NTS comes from intrinsic SP-containing neurons within the NTS and that SP released during isometric contractions excites an inhibitory pathway modulating baroreceptor input, thus contributing to an increase in mean blood pressure in the feline model. There is also an increasing body of work implicating SP in vagal afferents originating in the gastrointestinal tract and terminating in the brain stem. Picke ring et al. (20) suggest that noxious pinch, for example, releases SP within the NTS to selectively attenuate the cardiac vagal but not the sympathetic component of the baroreflex.

Furthermore, their findings confirm that baroreflex sympathetic and parasympathetic pathways may diverge and be independently controlled within the NTS.

Blockade of the NK₁ receptors in our study volunteers led to a small but significant attenuation of the baroreflex. This has not been demonstrated in human subjects to our knowledge and establishes a new mechanism for human baroreflex modification. Yet our results show only a modest attenuation of the BRS slope, without change in the sympathetic response to vasoactive drug, consistent with inhibition of the parasympathetic limb of the baroreflex. Since aprepitant crosses the blood-brain barrier, with access to NTS and other central sites of cardiovascular regulation, it is possible that alteration in central autonomic outflow might occur by mechanisms largely independent of baroreflex effects.

Body impedance data demonstrated a decrease in the resting systemic vascular resistance and a concomitant increase in the cardiac index, which allowed for stable arterial blood pressures with no change in the HR at rest or with HUT. In many physiological circumstances, changes in HR and CO track closely together, but not in all. Although there was an increase in resting stroke volume, we cannot say whether this was a direct effect of aprepitant. Whether blockade of NK₁ receptors could be useful in manipulation of physiological processes where lowering of systemic vascular resistance with an elevation in cardiac index may be beneficial, such as congestive heart failure, remains to be assessed.
Volunteers had decreased tilt tolerance during the aprepitant phase of the study. Two of the three patients experiencing the most consequential change began the study on placebo, so a training effect is not a likely explanation for this difference. Impaired orthostatic tolerance would be consistent with a lower systemic vascular resistance.

In our subjects, there was a significant increase in the HR on standing during the posture study. This change is typical of a hyperadrenergic state. The data that we have does not, however, demonstrate significant differences in plasma catecholamines at baseline. Although one might speculate that blockade of the NK1 receptor may produce a phenotype of the hyperadrenergic state, this was not supported by the totality of our data.

One interpretation of these results is that SP normally exerts a central enhancement of sympathoexcitatory outflow to some cardiovascular regions via the NK1, eliciting an increase in muscle sympathetic nerve activity and supporting sympathetic vascular tone.

With perturbations in condition, however, differences in components of the response emerge and can be detected, even though the final effect on blood pressure might be one of absent or minimal change. A number of visceral inputs into cardiovascular regulation, involving tachykinin-containing neurons, have long been recognized (12, 19, 25), some of which might be evoked by ischemia, stretch, or even osmotic stimuli (13, 21).

Limitations of this study include a predominance of male volunteers. The serum concentration of SP was not measured, and we do not know the exact effect of aprepitant on circulating levels of SP. Studies suggest that the dose of aprepitant administered in our study will have significant antagonism of the NK1 receptor, but this was not directly measured. This study was not powered to detect the small reduction in the sympathetic output of the baroreflex that was observed in this study.

In conclusion, this study implicates a role for SP in human cardiovascular regulation. SP has long been known as a modulator of neural pathways. This study provides evidence that a role for SP in the cardiovascular system emerges in the setting of perturbation of baseline variables by orthostatic stress.

REFERENCES


