Animal Model of Neuropathic Tachycardia Syndrome

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Abstract—Clinically relevant autonomic dysfunction can result from either complete or partial loss of sympathetic outflow to effector organs. Reported animal models of autonomic neuropathy have aimed to achieve complete lesions of sympathetic nerves, but incomplete lesions might be more relevant to certain clinical entities. We hypothesized that loss of sympathetic innervation would result in a predicted decrease in arterial pressure and a compensatory increase in heart rate. Increased heart rate due to loss of sympathetic innervation is seemingly paradoxical, but it provides a mechanistic explanation for clinical autonomic syndromes such as neuropathic postural tachycardia syndrome. Partially dysautonomic animals were generated by selectively lesioning postganglionic sympathetic neurons with 150 mg/kg 6-hydroxydopamine hydrobromide in male Sprague-Dawley rats. Blood pressure and heart rate were monitored using radiotelemetry. Systolic blood pressure decreased within hours postlesion (Δ>20 mm Hg). Within 4 days postlesion, heart rate rose and remained elevated above control levels. The severity of the lesion was determined functionally and pharmacologically by spectral analysis and responsiveness to tyramine. Low-frequency spectral power of systolic blood pressure was reduced postlesion and correlated with the diminished tyramine responsiveness (r=0.9572, P=0.0053). The tachycardia was abolished by treatment with the β-antagonist propranolol, demonstrating that it was mediated by catecholamines acting on cardiac β-receptors. Partial lesions of the autonomic nervous system have been hypothesized to underlie many disorders, including neuropathic postural tachycardia syndrome. This animal model may help us better understand the pathophysiology of autonomic dysfunction and lead to development of therapeutic interventions. (Hypertension. 2001;37:1357-1361.)

Key Words: blood pressure ■ rats ■ sympathectomy ■ tachycardia ■ heart rate

Blood pressure is exquisitely controlled through a balance among multiple regulatory systems, encompassing both rapid modulation by autonomic mechanisms and long-term regulation by the renin-angiotensin system and the kidney. Acute changes in arterial pressure are sensed by pressure and stretch receptors located in the aortic arch and the bifurcation of the carotids. Activation of baroreceptors through increased arterial pressure stimulates brain stem nuclei and leads to increased parasympathetic tone and decreased sympathetic outflow, whereas decreases in arterial pressure stimulate sympathetic outflow and decrease parasympathetic tone. Lesions of either the afferent or the efferent loop of the sympathetic outflow, whereas decreases in arterial pressure stimulate sympathetic outflow and decrease parasympathetic tone. Lesions of either the afferent or the efferent loop of the baroreflex can result in extreme volatility in blood pressure, leading to episodes of hypotension, hypertension, and syncope.1 Postural tachycardia syndrome (POTS)2 or orthostatic intolerance (OI) comprises diverse pathophysiologies affecting >500 000 Americans.3 The preeminent feature of OI is a tachycardia of >30 bpm on assumption of an upright posture. In addition, OI is characterized by an elevated supine heart rate, denervation supersensitivity, and elevated catecholamine levels.3 An association of OI with norepinephrine transporter deficiency has recently been reported by our group and may explain some cases of OI.4 One possible mechanism of idiopathic OI involves a partial lesion of efferent innervation of distal extremities.2,5,6 A form of OI with distal dysautonomia, neuropathic postural tachycardia syndrome (N-POTS), has recently been reported.7

Animal models reflecting loss of autonomic afferent and efferent innervation have been studied extensively. Multiple techniques for removal of efferent sympathetic innervation, sympathectomy, have been used.8 Previous studies in rats have yielded differing results concerning basal cardiovascular changes after sympathectomy, demonstrating increased, unchanged, or decreased heart rates.9-12

Data that support impaired sympathetic stimulation in human OI led us to test the hypothesis that selective loss of peripheral sympathetic innervation can result in an elevated heart rate.7 The aim of this study is to examine the cardiovascular consequences of a selective partial lesion of peripheral noradrenergic sympathetic nerves, with the hypothesis that under conditions of impaired sympathetic stimulation, a tachycardia may arise. Peripheral sympathetic nerves were selectively lesioned with the neurotoxin 6-hydroxydopamine (6-OHDA) in telemetered Sprague-Dawley rats. The use of
telemetry allowed examination of cardiovascular regulation using both pharmacological analysis and analysis of variability in a low-stress environment. Furthermore, this technique enabled data collection in the absence of anesthetic agents, which are known to decrease baroreflex sensitivity, decrease sensitivity to pressor agents, and decrease overall blood pressure variability. Responses to direct and indirect adrenergic agonists were examined during the recovery of sympathetic innervation.

Methods

Animals

The experimental protocol was approved by the Vanderbilt University Animal Care and Use Committee before implementation. Male Sprague-Dawley rats (Sasco, Omaha, Neb), 10 to 12 weeks of age at the onset of the experiment, were housed at 23°C with a 12-hour/12-hour light/dark cycle and were provided standard rodent chow and tap water ad libitum. At the end of the experimental protocol, animals were anesthetized with halothane and euthanized.

Drugs

6-OHDA hydrobromide, phenylephrine hydrochloride, (−)-isoproterenol hydrochloride, prazosin hydrochloride, propranolol hydrochloride, and tyramine were purchased from Sigma. 6-OHDA, prazosin, and propranolol were dissolved in 0.9% saline with 0.1% ascorbic acid and administered intravenously over 1 hour. Phenylephrine, isoproterenol, and tyramine were dissolved in 0.9% saline and administered as bolus intravenous injections.

Surgery

Rats were anesthetized with ketamine/acepromazine (75 mg/2.5 mg per kg). With the use of the aseptic technique, the abdominal aorta was exposed. The catheter attached to the radiotransmitter (TA11PA-C40, Data Sciences) was inserted into the abdominal aorta and the site was sealed with vascular adhesive (Vetbond, Data Sciences). The transmitter was then sutured to the inner abdominal wall, and the skin was closed with staples. Animals received a subcutaneous injection of ampicillin (60 mg/kg). To allow venous access, 5 to 7 days after implantation of the radiotransmitter, a heparinized catheter (PhysioCath, Data Sciences) was inserted into the jugular vein and tunneled under the skin to exit dorsally via a polyurethane button at the level of the neck. The venous access was filled with heparin (50 U, 50 μL) and flushed daily. Animals were allowed 3 to 4 days to recover before experimentation.

Sympathetic Lesion

Seven animals were treated with 200 μg/kg prazosin, 300 μg/kg propranolol, and 150 mg/kg 6-OHDA hydrobromide in 0.1% ascorbic acid/saline intravenously over 1 hour (total volume, 500 μL). Prazosin and propranolol were co-administered with 6-OHDA to attenuate the acute pressor and tachycardic effects accompanying 6-OHDA administration. Seven animals received vehicle alone (500 μL), consisting of 200 μg/kg prazosin and 300 μg/kg propranolol in 0.1% ascorbic acid/saline, administered over 1 hour.

Data Collection

All data were collected during the light cycle while the animal was resting quietly in its home cage. Beat-to-beat blood pressure data were acquired using telemetry and analyzed using the ART Gold software (Data Sciences). Heart rate and systolic blood pressure (SBP) were derived from the blood pressure waveform. To determine changes in basal blood pressure, heart rate, and spectral power before and after sympathetic lesioning, beat-to-beat blood pressure was collected continuously for 15 minutes in five 3-minute bursts while the animal was resting quietly. The average heart rate and SBP were determined from the average of the first 2 minutes of each burst, totaling 10 minutes of beat-to-beat data.

Spectral Analysis

Power spectral density, the variability at a specific frequency, was estimated by the fast Fourier transform–based Welch method.13 Intervals of 64 seconds, free from blood pressure changes due to behavior, ie, locomotor activity, eating, and grooming, were determined by linear regression to remove slow changes in the data, and a Hanning window was applied before spectral analysis. Frequency resolution was 0.015 Hz. The average power in the frequency ranges for low frequencies (LF, 0.25 to 0.6 Hz), which is variability that results from sympathetic activity, and high frequencies (HF, 1.0 to 2.0 Hz), which is variability due to parasympathetic activity and respiration, was calculated for each interval.12,14,15 The average of 5 spectra was used for the final determination of power spectral density.

Pharmacological Testing

To determine compensatory adaptations in adrenergic receptor sensitivity, the sensitivities to the α-selective agonist phenylephrine (5 μg/kg, 10 μg·kg⁻¹·min⁻¹) and to the nonselective β-agonist isoproterenol (0.05 μg/kg, 0.1 μg·kg⁻¹·min⁻¹) were examined. The severity of the sympathetic lesion was determined with the indirect noradrenergic agonist tyramine (500 μg/kg, 1 mg·kg⁻¹·min⁻¹). Sensitivity to agonists and tyramine was assayed both before 6-OHDA or vehicle administration and at 1, 4, 7, and 14 days after. Pharmacological agents were administered intravenously through PE-50 tubing attached to the catheter on the animal’s neck.

To determine cardiac catecholaminergic tone, a second group of animals, n=7, was lesioned and underwent pharmacological testing with phenylephrine and tyramine as described above. Following tyramine testing, the β-antagonist propranolol (2.5 mg/kg, 2.5 mg·kg⁻¹·min⁻¹) was administered. After propranolol administration, 15 minutes of cardiovascular data was collected as described above. The dose of propranolol used decreased the tachycardic response to 0.05 μg/kg isoproterenol by >90% 1 hour after administration (data not shown).

Statistics

Data are presented as the mean±SEM. Data were analyzed by repeated-measures ANOVA followed by the Dunnett posttest, which compared data in 6-OHDA–treated or vehicle-treated groups, or the Bonferroni posttest, which compared all data. P<0.05 was considered significant.

Results

The aim of this study was to test the hypothesis that selective and partial loss of peripheral sympathetic innervation can result in an elevated heart rate, which is similar to what is presumed to occur in some cases of neuropathic postural tachycardia syndrome. Sympathetic nerves were lesioned with the toxin 6-OHDA, and cardiovascular function was examined throughout the recovery process.

When the animals were resting quietly, SBP averaged 108±5 mm Hg (n=7) (Figure 1A). One day after lesioning with 6-OHDA, SBP decreased to 83±5 mm Hg (P<0.01), which was predicted from the loss of sympathetic tone. Over the next 4 days, SBP tended to return toward normal, reaching 99±3 mm Hg at the termination of the study 2 weeks postlesion. SBP of vehicle-treated animals did not change significantly during the study. Average heart rate under control conditions was 364±10 bpm (n=7) (Figure 1B). One day after 6-OHDA or vehicle administration, the heart rates of vehicle-treated and lesioned animals were unchanged. By 4 days postlesion, the mean heart rate of lesioned animals was significantly elevated, 401±16 bpm,
relative to prelesion ($P<0.05$), suggesting a compensatory mechanism to sustain blood pressure and cardiac output.

We chose to use spectral analysis as a nonpharmacological technique to assess sympathetic impairment after 6-OHDA. Global variability, or power, of a waveform can be expressed as the standard deviation. Spectral analysis is a technique that describes the frequencies in which the variability in a waveform can occur. Variability in discrete frequency domains have been determined to be associated with both sympathetic and parasympathetic control of the vasculature. LF variability, 0.25 to 0.6 Hz in rats, is known to be associated with sympathetic nerve firing, whereas HF variability, 1 to 2 Hz, is associated with parasympathetic activity and changes in intrathoracic pressure due to the mechanical act of respiration. Qualitatively, one can observe the loss of the 0.4 Hz oscillations in the SBP waveform and the resultant loss of LF variability in the SBP spectra (Figure 2A). In control animals, the LF power was $2.00 \pm 0.66$ mm Hg$^2$ and remained unchanged after vehicle treatment (Figure 2B). LF power decreased in lesioned animals from $2.46 \pm 0.79$ mm Hg$^2$ prelesion to $0.15 \pm 0.03$ mm Hg$^2$ 24 hours postlesion; then, LF power increased over the study to $0.55 \pm 0.40$ mm Hg$^2$ 2 weeks postlesion. HF power was unaffected by sympathectomy, demonstrating the specificity of the 6-OHDA lesion for sympathetic nerves.

To assess the effect of 6-OHDA on sympathetic nerve uptake and release mechanisms, animals were infused with 500 μg/kg tyramine. Tyramine is an indirect noradrenergic agonist, acting by stimulating release of norepinephrine from sympathetic nerve terminals. Before lesion, tyramine infusion resulted in a mean increase in SBP of 86 $\pm 5$ mm Hg (n=14) (Figure 3). One day after administration of 6-OHDA, the response to tyramine was reduced by 88% (11 $\pm 4$ mm Hg) in 6-OHDA–treated animals, whereas the response in vehicle-treated animals (2B, open bars) was unchanged. The pressor response to tyramine did tend to recover over time in the lesioned animals, reaching 51% of the prelesion response by 2 weeks postlesion. Tyramine responsiveness correlated with the LF spectral power ($r=0.9572$, $P=0.0053$).

The selective $\alpha_1$-agonist phenylephrine was used to examine the sensitivity of vascular $\alpha$-receptors. Before lesion, 5 μg/kg phenylephrine increased SBP by 46 $\pm 6$ mm Hg (Figure 4). One day postlesion, the sensitivity to phenylephrine was increased, resulting in a blood pressure increase of 63 $\pm 6$ mm Hg. The $\alpha_1$-supersensitivity persisted for 4 days after the lesion. The response to phenylephrine was unaffected by vehicle treatment. Baroreflex sensitivity was unchanged after both vehicle and 6-OHDA administration (data not shown).

Figure 1. Sympathetic lesioning with 6-hydroxydopamine results in a tachycardia. One day after lesioning sympathetic nerves with 6-OHDA, SBP was reduced by >20 mm Hg (filled bars, 1A), whereas heart rate had not changed from prelesion values. By 4 days postlesion, a time by which blood pressure had almost returned to basal, heart rate was elevated above prelesion values (filled bars, 1B). Heart rate tended to decrease in vehicle-treated animals (open bars, 1B) during the study, becoming significantly lower at the end of the study, relative to the prevehicle level. Data are shown as mean $\pm$ SEM and were analyzed by repeated-measures ANOVA with the Dunnett posttest. *$P<0.05$ vs prelesion, **$P<0.01$ vs prevehicle (n=7).

Figure 2. LF spectral power is reduced after sympathetic lesion. SBP spectra were generated before and at multiple time points after a sympathetic lesion. In a representative animal, the LF spectral peak associated with sympathetic activity can clearly be seen at 0.4 Hz (2A, dashed line). Sympathetic lesioning with 6-OHDA resulted in both an increased power in the very LF range and a dramatic absence of spectral power in the LF range, whereas HF power was unaffected (2A, solid line). Inset represents sample of data from which spectra were derived. Quantitatively, LF power is significantly decreased in animals treated with 6-OHDA (2B, closed bars) but not in vehicle-treated animals (2B, open bars). Data are shown as mean $\pm$ SEM and were analyzed by repeated-measures ANOVA with the Dunnett posttest. *$P<0.05$ vs prelesion, **$P<0.01$ vs prelesion (n=5).
Figure 3. Effects of sympathetic lesion on the response to tyramine. To assay the ability of sympathetic nerves to release noradrenaline, animals were administered the indirect noradrenergic agonist tyramine (500 µg/kg). The tyramine response is virtually absent 1 day postlesion, though it does return over the course of the study to 51% of control values, demonstrating functional sympathetic impairment. Data are shown as mean±SEM and were analyzed by ANOVA with the Tukey–Kranz posttest. **P<0.001 vs prelesion, #P<0.001 comparing 1 day postlesion to 14 days postlesion (n=7).

not shown). The nonselective β-agonist isoproterenol (0.05 µg/kg) was used to examine cardiac β1-receptor sensitivity. Isoproterenol increased heart rate by 109±7 bpm before 6-OHDA. The response to isoproterenol was unaffected by vehicle or 6-OHDA treatment (P=0.1779, ANOVA, data not shown).

To determine if the tachycardia present at 4 days postlesion was mediated by catecholamines, a second group of animals were lesioned and treated with the β-antagonist propranolol. Before lesion, propranolol significantly decreased heart rate from 360±8 bpm to 320±4 bpm (P<0.05, ANOVA). As expected, heart rate was elevated at 4 days postlesion (P<0.0001, ANOVA). The tachycardia was sensitive to propranolol, being reduced from to 424±15 bpm to 325±6 bpm after propranolol (P<0.0001, ANOVA).

Discussion

The goal of this study was to create a partial sympathetic lesion and to assess it as a model of neuropathic postural tachycardia syndrome. A selective peripheral noradrenergic lesion was induced with intravenous administration of the noradrenergic neurotoxin 6-OHDA. 6-OHDA enters nerve terminals via the norepinephrine transporter and induces free radical damage, resulting in destruction of the nerve terminal and release of catecholamines while sparing the cell body, thus allowing regeneration.16,17 6-OHDA has been used frequently as a tool to examine the role of the sympathetic nervous system, and the recovery of sympathetic nerves after 6-OHDA has been previously characterized.16,17 A postlesion tachycardia emerged coincident with sympathetic impairment.

We used analysis of variability as a nonpharmacological means to verify sympathetic impairment in these animals. Variability in the frequency range of 0.4 Hz is believed to be associated with central sympathetic stimulation or a resonance phenomenon of the baroreflex loop, dependent on sympathetic stimulation.18 Sympathectomy with 6-OHDA reduced spectral power in the LF range to 7% of prelesion values 1 day after 6-OHDA, whereas HF power was unaffected, which has been reported previously,19 demonstrating loss of sympathetic but not parasympathetic tone. The response to tyramine was decreased to 12% of prelesion values 24 hours after 6-OHDA and recovered to 51% of the prelesion response by 2 weeks, demonstrating the well-characterized functional recovery of sympathetic activity after 6-OHDA. The loss of both LF spectral power and tyramine responsiveness confirms that sympathetic nerves were impaired after 6-OHDA administration and remained impaired at 4 days postlesion, when the greatest elevation in heart rate was observed.

The elevation of heart rate might seem surprising in the context of sympathetic impairment. The tachycardia peaked at a time when sympathetic function was clearly impaired, which was determined by spectral analysis and tyramine responsiveness. Furthermore, administration of the β-antagonist propranolol abolished the postlesion tachycardia, suggesting that it is mediated by catecholamines that act through β-receptors. Presumably, the loss of cardiac sympathetic innervation after 6-OHDA administration would attenuate sympathetically mediated increases in heart rate. Previous studies examining tissue catecholamines after 6-OHDA or 6-hydroxydopa have demonstrated >90% depletion of cardiac norepinephrine within hours of toxin administration that remained for >1 week,19–21 supporting severe cardiac sympathetic impairment. Resistance of cardiac sympathetic innervation to the toxic effects 6-OHDA, as suggested by Kolibal-Pegher et al,22 may explain our results.

Alternatively, the lesion-induced tachycardia may result from increased release of epinephrine from the adrenal gland.16 Adrenal catecholamine levels are not appreciably depleted by 6-OHDA, and both plasma and urinary epinephrine levels have been reported to be elevated after sympathectomy.22

The demonstration that an elevation in heart rate resulted from damage to sympathetic nerves is of clinical relevance. Partial dysautonomia is believed to contribute to disorders such as N-POTS.5–7,23,24 It is believed that loss of distal sympathetic innervation may result in an exaggerated sympathetic response in innervated tissues such as the heart, resulting in a tachycardia. However, the 6-OHDA model of

Figure 4. Vascular α1-sensitivity is increased after 6-OHDA. The sensitivity of vascular α1-stimulation was examined using the selective agonist phenylephrine (5.0 µg/kg). Phenylephrine sensitivity was elevated 1 day and 4 days after 6-OHDA but had recovered toward prelesion sensitivity by 1 week (closed bars). Phenylephrine sensitivity was not significantly affected by vehicle treatment (open bars). Data are shown as mean±SEM and were analyzed by repeated-measures ANOVA with the Dunnett posttest. *P<0.05 vs prelesion, **P<0.01 vs prelesion (n=7).
dysautonomia differs from N-POTS in that cardiac sympathetic innervation is presumed to be largely intact in N-POTS, whereas in the 6-OHDA model, the heart is to some extent denervated. The animals in this study were examined while in a resting condition, and the results parallel the existence of a resting tachycardia seen in some patients with N-POTS.\textsuperscript{25} β-Adrenergic supersensitivity has been proposed as a mechanism of N-POTS, and indeed, β\(_2\)-supersensitivity to the chronotropic effects of isoproterenol has been reported, though it was not observed in this study.\textsuperscript{26,27} Abolition of the chronotropic effects of isoproterenol has been reported.

It might seem to be a limitation of our study that no experiments were conducted to stimulate orthostasis in rats. It is clear, however, in syndromes such as POTS, abnormal responses to many autonomic depressor stimuli occur, not just from the orthostatic stimulus that has given the disorder its name. Secondly, interpretation of cardiovascular changes due to an orthostatic stress would be complicated by the psychological stress that results from the physical act of inducing orthostasis in a conscious animal. This study aimed to parallel human studies in which patients were tested while resting quietly in a low-stress environment. Under these conditions, our data support the hypothesis that impaired sympathetic outflow can result in a tachycardia syndrome.

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