Published in final edited form as:

Ann Neurol. 2015 May ; 77(5): 743–752. doi:10.1002/ana.24377.

NOREPINEPHRINE DEFICIENCY WITH NORMAL BLOOD PRESSURE CONTROL IN CONGENITAL INSENSITIVITY TO PAIN WITH ANHIDROSIS

Lucy Norcliffe-Kaufmann, PhD¹, Stuart D. Katz, MD³, Felicia Axelrod, MD², and Horacio Kaufmann, MD²

¹Department of Physiology and Neurosciences, New York University, USA

²Department of Neurology, New York University, USA

³Department of Medicine, Division of Cardiology, New York University, USA

Abstract

Objective—Congenital insensitivity to pain with anhidrosis (CIPA) is caused by mutations in the *NKTR1* gene. This affects the development of nerve growth factor (NGF)-dependent neurons including sympathetic cholinergic neurons in the skin causing anhidrosis. Cardiovascular and blood pressure regulation appears normal, but the integrity of sympathetic adrenergic neurons has not been tested.

Methods—We examined the effect of posture on blood pressure, heart rate, plasma concentration of catecholamines, vasopressin, endothelin and renin activity in 14 patients with CIPA, 10 patients with chronically deficient sympathetic activity (pure autonomic failure) and 15 normal age-matched controls.

Results—In all 14 patients with CIPA plasma norepinephrine levels were very low or undetectable and failed to increase when upright, yet upright blood pressure was well maintained. Plasma epinephrine levels were normal and increased upright. Plasma renin activity also increased appropriately when upright and after furosemide-induced volume depletion. Nitric oxide-mediated endothelial function was intact. Patients with pure autonomic failure also had very low levels of plasma norepinephrine both supine and upright, but in contrast to patients with CIPA failed to maintain blood pressure upright.

Interpretation—The results indicate that post-ganglionic sympathetic neurons are severely depleted in CIPA, but chromaffin cells of the adrenal medulla are spared. This confirms the differential effect of NGF signaling for sympathetic neural and chromaffin cell development. The

Corresponding Author: Lucy Norcliffe-Kaufmann, PhD, Dysautonomia Center, 530 First Avenue, Suite 9Q, New York, NY, 10016, norcll01@nyumc.org, Phone: +1 212 263 7225.

Potential conflicts of interest: None

Author contributions: LJNK contributed to the conception and design of experiments, collection, analysis and interpretation of data, drafting the manuscript and revising it for critically important intellectual content. SK contributed to interpreting the data and revising the article for critically important intellectual content. FA contributed to collecting the data and drafting the article. HK contributed to the conception and design of experiments and revising the article for critically important content.

finding that patients with CIPA maintain blood pressure well on standing challenges current concepts of the role of norepinephrine in the regulation of arterial pressure.

Keywords

hereditary sensory and autonomic neuropathy type IV (HSAN-IV); congenital insensitivity to pain with anhidrosis (CIPA); norepinephrine; blood pressure; autonomic nervous system

INTRODUCTION

Congenital insensitivity to pain with anhidrosis (CIPA; OMIM 256800, also known as hereditary sensory and autonomic neuropathy [HSAN] type IV) was first described in children with mental retardation, insensitivity to pain, self-mutilating behavior and unexplained fevers.^{1–3} A cardinal feature of the disease is complete anhidrosis in response to thermal, emotional or direct stimulation of the skin.^{2, 3}

CIPA is inherited in an autosomal recessive manner and associated with missense, nonsense, and frame-shift mutations in the gene that encodes for the neurotrophic tyrosine kinase-1 receptor (TRKA). TRKA is normally expressed on specific neurons in the peripheral and central nervous system and forms part of the high affinity receptor for nerve growth factor.^{4, 5} Skin biopsies of patients with CIPA reveal that sweat glands are preserved,⁶ but lack sympathetic cholinergic innervation,⁷ thus the anhidrosis. Parasympathetic function appears to be normal, with an appropriate rise in heart rate following atropine infusion⁸ and preserved respiratory sinus arrhythmia.^{7, 9} Sympathetic adrenergic function is less well studied, but blood pressure regulation in these patients appears normal.^{9, 10} However, norepinephrine metabolism was found to be reduced^{2, 3, 8} and the patients' pressor response to exogenous norepinephrine was increased,^{2, 3} suggesting denervation super-sensitivity.

To directly test whether patients with CIPA have impaired sympathetic adrenergic function we examined and compared cardiovascular autonomic control and neuroendocrine responses in 14 patients with CIPA, 15 age-matched healthy controls and 12 patients with chronically impaired sympathetic adrenergic vasoconstrictor outflow (i.e., pure autonomic failure).¹¹ The results indicate that mutations of the NTRK1 gene and loss of TRKA-NGF signaling also hinder the development of sympathetic adrenergic vasoconstrictor neurons, and that the deficiency is likely compensated for by other blood pressure regulatory mechanisms.

METHODS

Patients

All study procedures were approved by the Institutional Review Board of New York University School of Medicine. All participants gave informed consent (or parental ascent when <18 years old). Fourteen patients with CIPA and genetic confirmation of a NTRK1 gene mutation (mean age 9±2 y, range: 1 – 31 y, 7 females) were studied. For comparison, 15 age-matched controls (age 10±2 years, range 2 months–17 years, 6 females) and 10 patients with chronically deficient sympathetic activity ("pure" autonomic failure, PAF, age 71±2 years, range 60–83 years, 5 females) were also studied.

Laboratory protocol

All subjects were studied in the morning after an overnight fast. In patients with chronic efferent autonomic failure, sympathomimetic drugs were withdrawn for 24-hours. Subjects first underwent clinical and neurological evaluations. On completion, an intravenous catheter was placed in the left cubital vein and subjects were transferred to the tilt table for instrumentation. To evaluate sympathetic activity, subjects performed the Valsalva maneuver with verbal coaxing to maintain an expiratory pressure of 40 mmHg for 15 seconds. To evaluate parasympathetic activity, breathing was paced at 6 breaths for 1 minute and electrocardiographic RR intervals recorded. To determine spontaneous heart rate variability, the lights were dimmed and RR intervals were recorded for 5 minutes while the patient was relaxed and silent with monitoring of respiratory rate (end-tidal CO₂ nasal cannula). To evaluate neuroendocrine responses to orthostatic stress, blood samples were taken to measure plasma concentration of catecholamines, vasopressin, renin activity, and aldosterone and endothelin levels while supine. Subjects were then tilted passively with footplate support to a 60-degree angle. After 10-minutes in the upright position, blood samples were repeated. Subjects were returned to the supine position and given 1-hour to rest. After recovering, brachial artery reactivity was measured in the supine position with high-resolution ultrasound imaging before and after 5 minutes of transient distal arterial occlusion (forearm cuff inflation to 50 mmHg above resting BP).^{12,13} To assess baroreflex mediated renin release, evaluations were repeated on a separate day in 2 siblings with CIPA 12-hours after diuretic administration (1 mg/kg furosemide). Room temperature in the laboratory was maintained at 25°C throughout.

Measurements

Beat-to-beat blood pressure was measured with finger plethysmography (Finometer/Finapres, the Netherlands). Intermittent readings were taken in the left arm with an automatic sphygmomanometer (Colin Press-Mate 7800, USA). Mean blood pressure was calculated as diastolic plus one third of the difference between systolic and diastolic. Electrocardiographic RR intervals were recorded from 3 precordial electrodes. All signals were acquired and digitized (PowerLab, 16SP Hardware, AD Instruments, New Zealand). Analysis was performed with LabChart software (AD Instruments). In brief, a 300-second segment of spontaneous breathing in the supine position was selected processed to detect RR intervals. The predominant influence of parasympathetic activity on heart rate variability was measured in the time domain by determining the percentage neighboring of RR intervals that differed by 50-msec or more (i.e., pnn50%) and the root mean square of successive differences in RR intervals (i.e., rMSSD).¹⁴ Heart rate variability in the frequency domain was evaluated using the power spectral in the high frequency (0.15 to <0.40 Hz) and low frequency (0.04 to <0.15 Hz) bands, following TaskForce guidelines.¹⁵ Heart rate variability during deep paced breathing was calculated from the average of 3 longest RR intervals during expiration divided by the average 3 shortest RR intervals during inspiration (i.e., expiratory:inspiratory ratio).¹⁴ Plasma catecholamines were measured using high-performance liquid chromatography with electrochemical detection. Radio-immunoassays were performed to measure plasma endothelin, vasopressin and renin activity (ARUP Laboratories, USA). Brachial artery flow velocity was measured using duplex ultrasound vascular imaging system and 11MHz transducer (axial resolution <0.1 mm).

Brachial artery diameter (trailing edge of anterior intima-lumen interface to leading edge of posterior lumen-intima interface) was measured in mm before inflation and one minute after release of at end-diastole (image analysis using Artery Measurement Systems, Sweden). Mean blood flow-velocity (velocity-time integral with correction for incident angle, cm/sec) was measured in before cuff inflation and 15 seconds after release. The percent change from pre-inflation brachial diameter to brachial artery diameter one minute after release of arterial occlusion was calculated.

Statistical Analysis

Responses to tilt were calculated as change from supine (baseline) and expressed as absolute or percentage change. Correlations between orthostatic blood pressures and renin release were assessed using non-linear regression analysis. Data were first tested for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Parametric and non-parametric evaluations were used as appropriate. Differences between groups were compared using 2-way ANOVA with multiple comparisons or Kruskal-Wallis test. Data was analyzed using Prism version 6.0 (GraphPad Software, Inc. 2014). All p values were 2-tailed and $p < 0.05$ was considered statistically significant. Data is expressed as mean $\pm$ SEM, unless otherwise stated.

RESULTS

Characteristics of the subjects

Table 1 summarizes the demographics and available information on the genotype in the patients with CIPA. All 14 CIPA patients had confirmation of NKTR1 gene mutations. All had characteristic clinical histories including hospitalizations due to hyperpyrexia; painless injuries including unrecognized bone fractures, and 8 had developed neuropathic (Charcot) joints. All of the children had cognitive/behavioral problems including; hyperactivity, impulsivity and learning difficulties. Cognitive status is summarized in the table. Neurological examination revealed elevated pain thresholds, but normal sense of touch, normal proprioceptive accuracy, preserved deep tendon reflexes and normal gait.

All of the patients with efferent autonomic failure fulfilled criteria for pure autonomic failure (PAF¹¹). All had symptomatic orthostatic hypotension, erectile dysfunction (5 men), constipation and variable degrees of anhidrosis as well as low circulating plasma concentration of norepinephrine and impaired parasympathetic cardiac control (Fig. 1–3). None had motor or cognitive deficits. The normal controls were age-matched to the CIPA patients and all healthy.

Cardiovascular autonomic responses

Autonomic control of heart rate—All patients with CIPA were in sinus rhythm and had normal heart rate variability (HRV) during spontaneous breathing (Fig. 1). Only four patients with CIPA were able to perform deep paced breathing adequately and all had normal expiratory:inspiratory ratios (Fig 1). HRV in the high frequency domain was higher in the patients with CIPA than in those with PAF (Fig. 1). Together these results all indicate preserved parasympathetic innervation of the heart.^{14, 16} LF:HF HRV ratio tended to be

higher in patients with CIPA suggesting a possible shift in sympathovagal balance toward parasympathetic modulation.¹⁵ As expected,^{14, 16} all patients with efferent autonomic failure (i.e., PAF) had blunted heart rate variability indicating impaired parasympathetic innervation of the heart (Fig. 1).

Baroreflex-mediated sympathetic activation—All control subjects had a normal overshoot in blood pressure after release of the Valsalva strain, confirming intact sympathetic activation. All patients with efferent autonomic failure had a pronounced fall in blood pressure during the straining phase (II) and no blood pressure overshoot after release (i.e., no phase IV), confirming efferent sympathetic failure. Despite their best efforts, only 2 CIPA patients were able to perform the Valsalva maneuver adequately. In these 2 patients, during the Valsalva strain (phase II), blood pressure fell (–21 and –61 mmHg) and RR intervals shortened appropriately (–61 and –233 ms, case 1 and 2 respectively). However, neither of the 2 CIPA patients showed an overshoot in blood pressure after release of the strain (i.e., no phase IV), a finding consistent with sympathetic noradrenergic impairment (data not shown).

Response to orthostatic stress—Patients with efferent autonomic failure had supine hypertension and as expected, their blood pressure fell progressively on head-up tilt (Figure 2a and 2b). Only half of these patients (5 of 10) were able to withstand the entire 10-minutes period of passive upright tilt. Ten patients with CIPA underwent passive upright tilt (the four youngest patients were unable to cooperate to perform the test). Patients with CIPA had normal blood pressures while resting supine (Figure 2a) and their blood pressure was well maintained on tilt with only a minimal transient fall in the first minutes (Figure 2b). All patients with CIPA tolerated the 10-minute tilt procedure without symptoms of orthostatic hypotension.

Patients with CIPA tended to have slightly slower resting heart rates than controls (Figure 2c). However, upon head-up tilt, they had a more pronounced initial increase in heart rate (Figure 2d), indicative of parasympathetic withdrawal.¹⁷ In contrast to normal controls, in the patients with CIPA, heart rate failed to increase further during prolonged orthostatic stress (2d), a response consistent with lack of sympathetic innervation of the sinoatrial node.¹⁸

Catecholamines—Supine plasma norepinephrine concentration was below detectable levels (<25 pg/ml) in three-quarters of patients with CIPA (<25 pg/ml, n=10) and well below those of controls in the remaining quarter (40 – 54 pg/ml, n=4; p<0.001, Fig. 3A). During upright tilt, norepinephrine levels failed to increase at all in the patients with CIPA (Fig. 3A), but surprisingly blood pressure was well maintained (2B). In patients with efferent autonomic failure, supine plasma norepinephrine was also low and failed to increase appropriately when upright, but contrary to the finding in patients with CIPA, they experienced a pronounced fall in blood pressure with symptoms of cerebral hypoperfusion (Fig. 3A). In contrast to norepinephrine, as shown in Fig 3B, supine resting epinephrine levels were normal in CIPA (CIPA 41±8 vs. Controls 54±22 pg/ml) and increased when upright, suggesting baroreflex-mediated epinephrine release.

Vasoactive peptides—To investigate compensatory mechanism by which patients with CIPA might maintain their blood pressure in the absence of norepinephrine, we explored the release of other vasoactive peptides. As shown in Figure 4, neither the upright release of vasopressin (Fig. 4A) or endothelin (Fig. 4B) was exaggerated in CIPA. In 3 patients aldosterone levels were similar supine and standing (15 ± 11 vs. 15 ± 11 ng/dL) and angiotensin-1-converting enzyme activity was unchanged (41 ± 25 vs. 46 ± 25 U/L). Plasma renin activity levels were normal in the supine position and increased appropriately when upright (Fig. 4C) and were positively correlated with the change in blood pressure (Figure 5A).

We additionally tested cardiovascular autonomic responses to intravascular volume depletion with furosemide (mean dose 40 mg) in 2 subjects. Following diuresis supine blood pressure was unchanged and surprisingly, blood pressure was still well maintained over 10-minutes of head up tilt. However, both patients were more tachycardic after diuresis; supine resting heart rates were 24 and 20 beats faster than without diuresis. Similarly, maximum heart rates during the tilt table were also higher, reaching 109 and 101 beats/min compared to 87 and 81 beat/min before diuresis. Despite the absence of norepinephrine, there was a robust increase in plasma renin activity following volume depletion in both patients, (Fig 5A). Norepinephrine levels remained undetectable supine and upright (Fig. 5B).

Endothelial function—Brachial artery reactivity in response to transient ischemia was assessed in 4 patients with CIPA, and all were within our laboratory normal range. The rest and peak mean blood flow velocity data (MBFV) are also within range of our laboratory normal.¹⁹ This observation suggests that local release of nitric oxide in response to increased blood flow and ischemia-mediated arteriolar vasodilation mechanisms in the skeletal muscle circulation are intact.

DISCUSSION

The main finding of our study is that patients with CIPA have very low or undetectable levels of circulating norepinephrine but normal epinephrine levels suggesting that lack of TRKA-NGF signaling affects the development of sympathetic adrenergic neurons but spares chromaffin cells in the adrenal medulla.

This selective impairment of NE with normal epinephrine is in line with previous reports showing reduced norepinephrine metabolism, but normal urinary output of epinephrine in patients with CIPA.^{2, 3, 8 10} Chromaffin cells are older phylogenetically and the findings in CIPA patients confirm animal data indicating that TRKA-NGF signaling is necessary for neuronal adrenergic but not required for chromaffin cell development.^{20, 21}

Despite the very low norepinephrine levels indicating impaired or absent sympathetic activity, CIPA patients regulate blood pressure well, contrary to patients with chronic autonomic failure and low levels of norepinephrine.^{11, 22}

Norepinephrine was undetectable in 10 of 14 patients with CIPA while in the remaining 4 only very low levels were detected (54, 40, 40 and 28 pg/ml). This may reflect phenotypic variability as a result of the different mutations spanning the NKTR1 gene.⁵ The cohort

with low but detectable levels of norepinephrine did not have a milder clinical phenotype nor did they share specific mutations.

The mechanism by which patients with CIPA maintain their blood pressure while standing in the absence of norepinephrine is intriguing. As epinephrine is an adrenergic agonist and raises blood pressure,^{23, 24} it is tempting to speculate that in the congenital absence of norepinephrine, adrenal epinephrine can “take over” sympathetic neurovascular signaling to increase peripheral vascular resistance and maintain blood pressure. Chromaffin cells in the adrenal medulla can secrete norepinephrine, which may account for the detectable norepinephrine levels in 4 of our patients.^{25, 26}

Parasympathetic function is intact in CIPA and withdraws appropriately when upright, although, it is unlikely that tachycardia can alone maintain blood pressure during orthostatic stress.^{27, 28} Reduced or absent nitric oxide synthesis cannot explain the lack of blood pressure fall, as vasodilation in response to transient ischemia was normal in the 4 patients in whom it was examined and none of the patients were hypertensive while supine.²⁹

The afferent arterioles have a rich network of sympathetic adrenergic neurons, which are thought to mediate renin secretion.^{30, 31} Renin secretion is inhibited by sympathetic ganglionic blockade³² and by renal sympathetic denervation.^{33, 34} The normal renin secretion during tilt in patients with CIPA indicates that renin release may be independent of sympathetic activation and norepinephrine release. It appears likely that other local and systemic factors can compensate for the lack of sympathetic nerves to regulate renin secretion. The robust renin release following diuretics suggests that macula densa mechanisms are intact in these patients.^{35, 36} Moreover, a fall in perfusion pressure at the afferent arteriole following diuretic use and upon standing can also stimulate renin secretion, independent of sympathetic activation of beta-adrenergic receptors.³⁶ In addition, prostaglandin secretion, nitric oxide release, epinephrine secretion and intracellular signaling pathways could potentially play a role preserving renin release in the absence of sympathetic fibers.^{35, 36}

The cardiovascular phenotype of CIPA (HSAN IV) with normal afferent and abnormal efferent autonomic neurons, is a “mirror image” to that of HSAN III patients who have normal efferent and abnormal afferent neurons. These distinctive phenotypes have implications for our understanding of the development of the peripheral autonomic nervous system. HSAN-III is caused by a mutation in the elongator-1 protein,³⁷ which impairs the development of afferent baroreceptor neurons, but spares efferent sympathetic neurons.^{22, 38–40}

Our findings in CIPA show the reverse; afferents baroreceptor nerves are functional, but efferent sympathetic nerves are absent. This suggests that elongator-1 is critical for the development of afferent nerves,^{22, 41} whereas TRKA-NGF receptor signaling is necessary for the survival of efferent sympathetic neurons.^{42, 43}

Neuropathology studies in CIPA are scarce. The spinothalamic tracts and descending autonomic pathways from the medulla and pons appear to be spared⁴⁴. Peripheral unmyelinated fibers are virtually absent in the sural nerve⁴⁵ and sympathetic (cholinergic)

fibers innervating the sweat glands are not found.^{7, 45} Our findings suggest that sympathetic post-ganglionic adrenergic neurons are also absent.

Neural crest cell from the trunk in the embryo develop into sympathetic postganglionic neurons and chromaffin cells. Whether they share a same progenitor cell line is unknown. The development of chromaffin cells does not require TRKA-NGF signaling.^{20, 21}

Alterations in venous capacitance, intrinsic myogenic reflexes in vascular smooth muscle, vascular responses to epinephrine or as yet undiscovered reflexes may be involved in increasing venous return to the heart. Unraveling the mechanisms that allow patients with CIPA to maintain blood pressure upright in the absence of norepinephrine has implications for the treatment of other common disorders of blood pressure control.

Acknowledgements

This work was supported by grants from the Dysautonomia Foundation, Inc and from the National Institutes for Health (U54NS065736). We wish to thank Dr. Jelani Qurat-Ul-Ain for her help acquiring the forearm mediated flow responses and Dr. Juan Oliver for his helpful comments.

REFERENCES

1. Gillespie JB, Perucca LG. Congenital generalized indifference to pain (congenital analgia). *Am J Dis Child*. 1960 Jul;100:124–126. [PubMed: 13850089]
2. Swanson AG. Congenital insensitivity to pain with anhidrosis. A unique syndrome in two male siblings. *Arch Neurol*. 1963 Mar;8:299–306. [PubMed: 13979626]
3. Pinsky L, DiGeorge AM. Congenital familial sensory neuropathy with anhidrosis. *J Pediatr*. 1966 Jan; 68(1):1–13. [PubMed: 4158991]
4. Shatzky S, Moses S, Levy J, et al. Congenital insensitivity to pain with anhidrosis (CIPA) in Israeli-Bedouins: genetic heterogeneity, novel mutations in the TRKA/NGF receptor gene, clinical findings, and results of nerve conduction studies. *American journal of medical genetics*. 2000 Jun 19; 92(5):353–360. [PubMed: 10861667]
5. Indo Y, Tsuruta M, Hayashida Y, et al. Mutations in the TRKA/NGF receptor gene in patients with congenital insensitivity to pain with anhidrosis. *Nat Genet*. 1996 Aug; 13(4):485–488. [PubMed: 8696348]
6. Langer J, Goebel HH, Veit S. Eccrine sweat glands are not innervated in hereditary sensory neuropathy type IV. An electron-microscopic study. *Acta Neuropathol*. 1981; 54(3):199–202. [PubMed: 6167137]
7. Nolano M, Crisci C, Santoro L, et al. Absent innervation of skin and sweat glands in congenital insensitivity to pain with anhidrosis. *Clin Neurophysiol*. 2000 Sep; 111(9):1596–1601. [PubMed: 10964070]
8. Vassella F, Emrich HM, Kraus-Ruppert R, Aufdermaur F, Tonz O. Congenital sensory neuropathy with anhidrosis. *Arch Dis Child*. 1968 Feb; 43(227):124–130. [PubMed: 4171106]
9. Ohto T, Iwasaki N, Fujiwara J, et al. The evaluation of autonomic nervous function in a patient with hereditary sensory and autonomic neuropathy type IV with novel mutations of the TRKA gene. *Neuropediatrics*. 2004 Oct; 35(5):274–278. [PubMed: 15534759]
10. Loewenthal N, Levy J, Schreiber R, et al. Nerve growth factor-tyrosine kinase A pathway is involved in thermoregulation and adaptation to stress: studies on patients with hereditary sensory and autonomic neuropathy type IV. *Pediatric research*. 2005 Apr; 57(4):587–590. [PubMed: 15695606]
11. Freeman R, Wieling W, Axelrod FB, et al. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Clin Auton Res*. 2011 Apr; 21(2):69–72. [PubMed: 21431947]

12. Harris RA, Nishiyama SK, Wray DW, Richardson RS. Ultrasound Assessment of Flow-Mediated Dilation. *Hypertension*. 2010 May; 55(5):1075–1085. [PubMed: 20351340]
13. Zheng H, Cable R, Spencer B, Votto N, Katz SD. Iron stores and vascular function in voluntary blood donors. *Arteriosclerosis, thrombosis, and vascular biology*. 2005 Aug; 25(8):1577–1583.
14. Freeman R, Chapleau MW. Testing the autonomic nervous system. *Handbook of clinical neurology*. 2013; 115:115–136. [PubMed: 23931777]
15. Task force of the European Society of Cardiology. Heart Rate Variability: Standards of measurement, physiological interpretation, and clinical use. *circulation*. 1996; 93:1043–1065. [PubMed: 8598068]
16. Cygankiewicz I, Zareba W. Heart rate variability. *Handbook of clinical neurology*. 2013; 117:379–393. [PubMed: 24095141]
17. Guzzetti S, Borroni E, Garbelli PE, et al. Symbolic dynamics of heart rate variability: a probe to investigate cardiac autonomic modulation. *Circulation*. 2005 Jul 26; 112(4):465–470. [PubMed: 16027252]
18. Tellez MJ, Norcliffe-Kaufmann LJ, Lenina S, Voustianiouk A, Kaufmann H. Usefulness of tilt-induced heart rate changes in the differential diagnosis of vasovagal syncope and chronic autonomic failure. *Clin Auton Res*. 2009 Dec; 19(6):375–380. [PubMed: 19834645]
19. Jelani QU, Norcliffe-Kaufmann L, Kaufmann H, Katz SD. Vascular Endothelial Function and Blood Pressure Regulation in Afferent Autonomic Failure. *American journal of hypertension*. 2014 Aug 15.
20. Schober A, Wolf N, Huber K, et al. TrkB and neurotrophin-4 are important for development and maintenance of sympathetic preganglionic neurons innervating the adrenal medulla. *J Neurosci*. 1998 Sep 15; 18(18):7272–7284. [PubMed: 9736648]
21. Schober A, Minichiello L, Keller M, et al. Reduced acetylcholinesterase (AChE) activity in adrenal medulla and loss of sympathetic preganglionic neurons in TrkA-deficient, but not TrkB-deficient, mice. *J Neurosci*. 1997 Feb 1; 17(3):891–903. [PubMed: 8994044]
22. Norcliffe-Kaufmann L, Axelrod F, Kaufmann H. Afferent baroreflex failure in familial dysautonomia. *Neurology*. 2010 Nov 23; 75(21):1904–1911. [PubMed: 21098405]
23. Norcliffe-Kaufmann L, Gonzalez-Duarte A, Martinez J, Kaufmann H. Tachyarrhythmias with elevated cardiac enzymes in Munchausen syndrome. *Clin Auton Res*. 2010 Apr 28.
24. Barbe P, Galitzky J, Thalamos C, et al. Increase in epinephrine-induced responsiveness during microgravity simulated by head-down bed rest in humans. *Journal of applied physiology*. 1999 Nov; 87(5):1614–1620. [PubMed: 10562599]
25. Ito Y, Fujimoto Y, Obara T. The role of epinephrine, norepinephrine, and dopamine in blood pressure disturbances in patients with pheochromocytoma. *World journal of surgery*. 1992 Jul-Aug; 16(4):759–763. discussion 63–4. [PubMed: 1413846]
26. Tischler AS, Pacak K, Eisenhofer G. The adrenal medulla and extra-adrenal paraganglia: then and now. *Endocrine pathology*. 2014 Mar; 25(1):49–58. [PubMed: 24362581]
27. Jordan J, Shannon JR, Diedrich A, Black BK, Robertson D. Increased sympathetic activation in idiopathic orthostatic intolerance: role of systemic adrenoreceptor sensitivity. *Hypertension*. 2002 Jan; 39(1):173–178. [PubMed: 11799098]
28. El-Bedawi KM, Wabha MA, Hainsworth R. Cardiac pacing does not improve orthostatic tolerance in patients with vasovagal syncope. *Clin Auton Res*. 1994; 4(5):233–237. [PubMed: 7888741]
29. Spieker LE, Flammer AJ, Luscher TF. The vascular endothelium in hypertension. *Handbook of experimental pharmacology*. 2006; 176(Pt 2):249–283. [PubMed: 16999229]
30. DiBona GF. Neural regulation of renal tubular sodium reabsorption and renin secretion. *Federation proceedings*. 1985 Oct; 44(13):2816–2822. [PubMed: 2995141]
31. Johns EJ, Kopp UC, DiBona GF. Neural control of renal function. *Comprehensive Physiology*. 2011 Apr; 1(2):731–767. [PubMed: 23737201]
32. Kaneko Y, Takeda T, Ikeda T, et al. Effect of ganglion-blocking agents on renin release in hypertensive patients. *Circ Res*. 1970 Jul; 27(1):97–103. [PubMed: 4393196]
33. Arnold AC, Okamoto LE, Gamboa A, et al. Angiotensin II, independent of plasma renin activity, contributes to the hypertension of autonomic failure. *Hypertension*. 2013 Mar; 61(3):701–706. [PubMed: 23266540]

34. Gulati V, White WB. Review of the state of renal nerve ablation for patients with severe and resistant hypertension. *Journal of the American Society of Hypertension : JASH*. 2013 Nov-Dec; 7(6):484–493. [PubMed: 23953998]
35. Persson PB. Renin: origin, secretion and synthesis. *J Physiol*. 2003 Nov 1; 552(Pt 3):667–671. [PubMed: 12949225]
36. Schweda F, Kurtz A. Regulation of renin release by local and systemic factors. *Reviews of physiology, biochemistry and pharmacology*. 2011; 161:1–44.
37. Slaughter SA, Blumenfeld A, Gill SP, et al. Tissue-specific expression of a splicing mutation in the IKBKAP gene causes familial dysautonomia. *Am J Hum Genet*. 2001 Mar; 68(3):598–605. [PubMed: 11179008]
38. George L, Chaverra M, Wolfe L, et al. Familial dysautonomia model reveals Ikbkap deletion causes apoptosis of Pax3+ progenitors and peripheral neurons. *Proc Natl Acad Sci U S A*. 2013 Nov 12; 110(46):18698–18703. [PubMed: 24173031]
39. Norcliffe-Kaufmann L, Martinez J, Axelrod F, Kaufmann H. Hyperdopaminergic crises in familial dysautonomia: a randomized trial of carbidopa. *Neurology*. 2013 Apr 23; 80(17):1611–1617. [PubMed: 23553478]
40. Norcliffe-Kaufmann LJ, Axelrod FB, Kaufmann H. Cyclic vomiting associated with excessive dopamine in Riley-day syndrome. *J Clin Gastroenterol*. 2013 Feb; 47(2):136–138. [PubMed: 22739220]
41. Gutierrez JV, Norcliffe-Kaufmann L, Kaufmann H. Brainstem reflexes in patients with familial dysautonomia. *Clin Neurophysiol*. 2014 Jul 3.
42. Majdan M, Walsh GS, Aloyz R, Miller FD. TrkA mediates developmental sympathetic neuron survival in vivo by silencing an ongoing p75NTR-mediated death signal. *The Journal of cell biology*. 2001 Dec 24; 155(7):1275–1285. [PubMed: 11756477]
43. Rafel E, Alberca R, Bautista J, Navarrete M, Lazo J. Congenital insensitivity to pain with anhidrosis. *Muscle Nerve*. 1980 May-Jun; 3(3):216–220. [PubMed: 6154886]
44. Swanson AG. Congenital insensitivity to pain with anhidrosis. *Arch Neurol*. 1963; 8:299–306. [PubMed: 13979626]
45. Goebel HH, Veit S, Dyck PJ. Confirmation of virtual unmyelinated fiber absence in hereditary sensory neuropathy type IV. *J Neuropathol Exp Neurol*. 1980 Nov; 39(6):670–675. [PubMed: 6161237]

Figure 1. Intact parasympathetic control of heart rate in CIPA

A) Shows time domain analysis of heart rate variability using pnn50 in a 300-second recording of RR intervals during spontaneous breathing. B) Shows time domain analysis of heart rate variability using rMSSD in the same recording. C) Shows the ratio between RR interval lengthening during expiration and shortening during inspiration with breathing paced at 6 breaths/min. D) Shows HRV in the high frequency domain. E) Shows HRV in the low frequency domain. Note, measurements reflect intact parasympathetic modulation of heart rate in patients with CIPA. F) Shows the ratio between low frequency and high power derived from spectral measures of heart rate variability in the same 300-second recording. The trend ($p=0.061$) towards a higher LF:HF ratio indicates a possible shift in sympathovagal-balance towards predominance of parasympathetic influences on the heart. Statistical differences determined with ANOVA and multiple comparisons. Key: * $p<0.05$, ** $p<0.01$, **** $p<0.0001$. Pnn50, percentage of adjacent RR intervals that differ by 50-msec or more; rMSSD, mean root square of differences between adjacent RR intervals; E:I, expiratory:inspiratory ratio; HF, high frequency heart rate variability; LF, low frequency heart rate variability; CIPA, congenital insensitivity to pain with anhidrosis; PAF, pure (efferent) autonomic failure.

Figure 2. Blood pressure and heart rate response to passive upright tilt

2A) Shows supine mean blood pressure 2B) shows the mean orthostatic change in blood pressure at min 1, 3 and 10 of passive upright tilt. Note, relatively well maintained blood pressure in patients with CIPA in comparison to those with efferent pure autonomic failure (PAF). 2C) Shows supine heart rate. 2D) Shows change in heart at min 1, 3 and 10 of passive upright tilt. Note, initial tachycardia on upright tilt in patients with CIPA that fails to continue increasing throughout prolonged tilt. Statistical differences determined with ANOVA and multiple comparisons. Key: * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. CIPA, congenital insensitivity to pain with anhidrosis; PAF, pure (efferent) autonomic failure; HR, heart rate; MBP, mean blood pressure.

Figure 3. Catecholamines supine and standing

3A) Shows plasma [NE] supine. <25 pg/ml is below the limit of detection B) Shows percentage change in plasma [NE] after 10 minutes of upright tilt. Note, extremely low or undetectable plasma norepinephrine levels in patients with CIPA and failure to increase norepinephrine when upright. C) Shows plasma [EPI] in the supine position. <10 pg/ml is below the limit of detection. D) Shows percentage change in [EPI] after 10 minutes of head-up tilt. Note, the normal supine epinephrine levels that increase when tilted upright in the patients with CIPA. Statistical differences determined with ANOVA and multiple

comparisons. Key: *** $p < 0.001$, **** $p < 0.0001$. [NE], plasma concentration of norepinephrine; [EPI], plasma concentration of epinephrine; CIPA, congenital insensitivity to pain with anhidrosis; PAF, pure (efferent) autonomic failure.

A) Vasopressin **B) Endothelin** **C) Renin** **Figure 4. Vasoactive peptide release with upright passive tilt**

A) Left bar chart shows absolute plasma vasopressin levels when supine and right bar chart shows change in plasma vasopressin levels after 10 minutes of head-up tilt. B) Left bar chart shows absolute plasma endothelin levels while supine and right bar chart shows change in plasma endothelin levels with tilt. Note, neither vasopressin or endothelin release was exaggerated in patients with CIPA compared with controls. C) Right bar chart shows supine plasma renin activity and left chart shows change in plasma renin activity was preserved in the patients with CIPA. Statistical differences determined with ANOVA and multiple

comparisons. Key: * $p < 0.05$, ** $p < 0.01$. [AVP], plasma concentration of arginine vasopressin; PRA, plasma renin activity; CIPA, congenital insensitivity to pain with anhidrosis. PAF, pure (efferent) autonomic failure.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 5. Renin release in patients with CIPA

Data indicates intact renin secretion after intravascular volume depletion in the absence of norepinephrine. A) Shows the significant correlation between change in plasma renin activity and change in MBP at 10 minutes of passive upright tilt in patients with CIPA ($n=8$, upright intravenous line failed in 2 patients). Data indicates intact baroreflex-mediated renin release despite the absence of norepinephrine. B) Shows plasma renin activity and norepinephrine levels in two siblings with CIPA (boy age 10-y and girl age 13-y) in the supine position at baseline. Arrow indicates administration of furosemide. Twelve hours after furosemide, there was a robust increase in renin activity supine and a further increase after 10 minutes of upright tilt. Norepinephrine levels remained undetectable throughout (<25 pg/ml). Key: ** $p < 0.01$. PRA, plasma renin activity; [NE], plasma concentration of norepinephrine; MBP, mean blood pressure.

TABLE

Genotype and clinical characteristics of the patients with CIPA

Case	Mutation	Origin	Consanguinity	Fracture Hx	Hyperpyrexia	Charcot joints	Cognition
19/F	Heterozygous, Novel	Greek	+	+	+	+	IQ:76, learning disabled
14/M	Homozygous ^a	Palestine	+	+	+	+	Learning disabled
19/M	Homozygous ^a	Palestine	+	+	+	+	IQ: 48, mental retardation
7/M	Confirmed ^c	Asia	-	+	+	+	IQ: 65, Learning disabled
10/M	Heterozygous, splice	USA	-	+	+		Mental retardation
1/F	Homozygous, missense	Middle East	+	+	+		Developmental delay
8/M	Heterozygous, duplication	Adopted	Unknown	+	+		Learning disabled
7/M	Confirmed ^c	USA/Mexican	-	+	+	+	Mental retardation
2/F	Heterozygous, deletion	European/USA	-	+	+		Developmental delay
0.6/F	Heterozygous, splice	Asia	-		+		Developmental delay
13/M	Homozygous, deletion ^b	Middle East	+	+	+	+	Learning disabled
10/F	Homozygous, deletion ^b	Middle East	+	+	+		Learning disabled
5/F	Confirmed ^c	Adopted	+	+	Unknown	+	Mental retardation
29/F	Heterozygous, Novel	European	-	+	+	+	Learning disabled

^aDenotes sibling set with confirmation of TrkA mutation without further details,^bDenotes sibling set,^cDenotes case with confirmation of the TrkA mutation without further details. Mental status was assessed by trained pediatrician (FBA) or based on school records. Age in years. F, female; M, male. + yes, - no