Inactivation of one copy of the mouse neurotrophin-3 gene induces cardiac sympathetic deficits

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Departments of 1Anatomy and 2Physiology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272-0095; 3Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan 48201; and 4Department of Neurology, Boston University School of Medicine, Boston Massachusetts 02118

Story, Gina M., Stephen E. DiCarlo, David W. Rodenbaugh, Dean E. Dluzen, Jan Kucera, Michael B. Maron, and Jon M. Walro. Inactivation of one copy of the mouse neurotrophin-3 gene induces cardiac sympathetic deficits. Physiol Genomics 2: 129–136, 2000.—Whether two copies of the neurotrophin-3 (NT3) gene are necessary for proper development of cardiac sympathetic innervation was investigated in mice carrying a targeted inactivation of the NT3 gene. Heterozygous (+/−) and null (−/−) mutant mice had fewer stellate ganglion neurons than did wild-type (+/+) mice at postnatal day 0 (P0 or birth), and this deficit was maintained between adult (P60) +/+− and −/+ mice. The sympathetic innervation of the heart matured postnatally in +/+− and −/+ mice. Tyrosine hydroxylase (TH)-positive axons were restricted largely to the epicardium at P0, were concentrated around large blood vessels in the myocardium at P21, and were present among cardiac myocytes at P60. Cardiac norepinephrine (NE) concentrations paralleled the growth of sympathetic axons into the heart. NE concentrations were equivalent among +/+−, +/+−, and −/+ mice at birth, but differences between +/−− and +/−+ mice increased with age. Adult +/−− mice also exhibited lower resting heart rates and sympathetic tonus than +/+− mice. Thus deletion of one copy of the NT3 gene translates into anatomical, biochemical, and functional deficits in cardiac sympathetic innervation of postnatal mice, thereby indicating a gene-dosage effect for the NT3 gene.

sympathetic nervous system; norepinephrine; cardiac function; knockout mice

NEUROTROPHINS CONSTITUTE a family of four structurally similar trophic factors required for the proliferation, differentiation, and survival of different classes of vertebrate neurons (17). Sympathetic and sensory neurons require two neurotrophins, neurotrophin-3 (NT3) and nerve growth factor (NGF), during development, as evidenced by substantial deficits in these neuron populations in null mutant (−/−) mice, which lack either the NT3 or NGF genes (2, 4, 8, 9).

Studies that utilized targeted inactivation of neurotrophin genes to investigate the roles of neurotrophins in vivo focused primarily on the impact of a complete absence of these factors on neuron populations in −/− mice (4, 8, 9). A major limitation of this type of study is that −/− mice die at birth or shortly thereafter due to pleiotropic effects on different organs or a failure to thrive (5, 8). Neurotrophins are also required by mature, fully differentiated neurons (24, 29, 30). Therefore, the premature mortality of −/− mice limits their usefulness for investigating the function of the neurotrophins in postnatal mice.

Heterozygous mutant (+/−) NT3 mice, in which a single copy of the NT3 gene has been inactivated, are visually indistinguishable from wild-type (+/+−) mice and reproduce well (8). Few studies have closely examined the phenotype of NT3 mice. However, while conducting a detailed analysis of −/− NT3 mice, Ernfors et al. (8) noted that +/− NT3 mice exhibit a significant loss of sensory neurons. Similarly, deficits in sensory (4) and sympathetic (2) neurons occur in NGF +/+− mice.

This study addressed whether two copies of the NT3 gene are necessary for proper pre- and postnatal development of the sympathetic innervation of the heart by systematic analysis of anatomical, biochemical, and physiological parameters of sympathetic innervation in NT3 +/− mice at birth, weaning, and adulthood. We show that deletion of a single copy of the NT3 gene results in quantifiable structural and functional deficits in the sympathetic innervation of the heart of postnatal mice, thereby indicating a gene-dosage effect for the NT3 gene.

METHODS

Transgenic mice. The NT3 mutant strain used was developed on a Balb/c 129 background by insertion of a partially deleted coding exon for the NT3 gene (8). The Animal Care and Use Committees at Northeastern Ohio Universities and Wayne State University Colleges of Medicine, in accordance with NIH Guidelines for the Care and Use of Laboratory Animals, approved all experimental procedures. Mice were housed in plastic cages, fed and watered ad libitum, and maintained on a 12:12-h light-dark cycle. NT3 +/− mice were mated to produce offspring. Mice were genotyped as +/−−, +/−+, or −/− by PCR amplification and 2% agarose gel electrophoresis of tail DNA (Fig. 1). Oligonucleotide primers used for genotyping mice were dMR130, dMR131, and dMR132. Two separate reactions were run for each sample using dMR130 in combination with dMR131 and dMR132,
which amplified products from the wild-type and targeted alleles, respectively (J. Jackson Laboratory IMR protocol).

Heart/body weight ratios. Heart/body weight ratios were calculated to determine whether the hearts of +/− or −/− mice were atrophied or hypertrophied relative to +/+ mice. Mice were weighed just prior to euthanasia. Hearts were excised, drained of excess blood, and weighed immediately following euthanasia.

Neuron counts. The number of neurons in the left stellate ganglion (LSG) of +/+, +/-, and −/− newborn (postnatal day 0 or P0) mice and +/- and +/- weanling (P21) and adult (P60) mice were counted to estimate the number of cardiac sympathetic neurons. The thoraxes of P0 and P21 mice were immersed overnight in Bouin’s fixative, transferred to 70% ethanol, and processed for paraffin embedding. Serial sections were cut at 6 µm, stained with hematoxylin and eosin, and mounted on glass slides. Only nucleated cells with a prominent nucleolus (a feature of neurons in the LSG) were counted at 30-µm intervals throughout the ganglion. The LSG of adult mice were dissected and fixed in cold 2% paraformaldehyde-2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer. After postfixation in 1% OsO4, ganglia were dehydrated and embedded in Embed 812. Serial 1-µm sections were collected at 50-µm intervals throughout the ganglion, and neurons were counted using the same criteria as for P0 and P21 mice. The average nucleolar diameter was estimated from 20 randomly sampled nuclei in a series of serial 1-µm sections. The total number of neurons in the LSG of P0, P21, and P60 mice was estimated by a classic technique. The counts of neurons in sections were summed, multiplied by the interval length, and divided by the mean nucleolar length (27).

TUNEL assay and tyrosine hydroxylase immunocytochemistry. A terminal transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay was used to identify apoptotic cells in the LSG from two mice of each genotype on P0, and data were correlated with anti-tyrosine hydroxylase (anti-TH) immunocytochemistry on adjacent sections to demonstrate that apoptotic cells were sympathetic neurons. Tissues were fixed in 4% paraformaldehyde for 24 h at 4°C and 24 h at 25°C, embedded in paraffin, sectioned sagittally at 6 µm, and mounted on glass slides. Sections were transferred through xylene and descending concentrations of ethanol, then rehydrated in PBS. TUNEL sections were treated for 20 min with 25 µg/ml proteinase K in PBS. Positive controls were then exposed to 1 µg/ml DNase I for 5 min. Endogenous peroxidase activity was blocked by incubating the sections in 3% H2O2 for 5 min. Sections were rinsed in 0.5 M Tris, pH 7.5, 50 mM MgCl2, 0.6 mM mercaptoethanesulfonic acid, and 0.5 mg/ml BSA for 10 min, then incubated at 37°C for 2.5 h in the same buffer containing 2 µl Klenow enzyme (300 U/ml) and 20 µl biotinylated nucleotide mix. Negative controls were incubated in the same mixture without the Klenow enzyme. Rinsing in 3 M NaCl and 300 mM sodium citrate solution for 5 min terminated the reaction. After a rinse in distilled H2O, sections were incubated with a 1:500 mixture of streptavidin-peroxidase conjugate and strept-avidin-peroxidase diluent for 10 min. Subsequently, sections were stained with blue label dye (Trevigen, Gaithersburg, MD) for 10 min, dehydrated with an ethanol series, cleared with xylene, and coverslipped with mounting medium.

Adjacent sections of the thoracic regions of +/+ and −/− mice on P0 and +/+ and −/+ mice on P21 and P60 were fixed, embedded in paraffin, sectioned, and processed for anti-TH immunocytochemistry according to the protocol used for identifying sympathetic neurons in the LSG.

Noradrenaline measurement. The extent of cardiac sympathetic innervation was estimated by measuring noradrenaline (NE) concentration in the heart. At P0, P21, and P60, mice were weighed and then euthanized by decapitation (P0) or cervical dislocation (P21, P60). Hearts were excised and in 10–20 mg of the free wall of the left ventricle of P21 or P60 mice were immersed in cold 0.1 M perchloric acid. Tissue samples were homogenized in a glass-walled tissue grinder and centrifuged at 3,000 rpm for 5 min at 4°C to pellet cell debris. The supernatant was aspirated through a 0.45-µm filter, and the concentration of NE in the filtrate of each sample was analyzed by HPLC.
Instrumented, conscious, unrestrained mice were placed in a large Plexiglas box (30.5 cm³) and allowed to adapt to the laboratory environment for 1 h. After baseline hemodynamic measurements were obtained, HR, AP, and MAP responses to cardiac autonomic (muscarnic-cholinergic and β₁-adrenergic) blockade were measured. Cardiac muscarinic-cholinergic receptor blockade was achieved by infusion of the non-selective receptor antagonist, methylatropine (MA, 3 mg/kg) through the carotid arterial catheter. HR response to MA peaked 10–15 min after infusion; therefore, this interval was selected as a standard before HR was measured. Cardiac β₁-adrenergic receptor blockade was achieved by infusion of the selective β₁-adrenergic antagonist, metoprolol (MT, 10 mg/kg) into the catheter. MT was infused 15 min after MA, and HR was measured again after 15 min. Intrinsic heart rate (HRᵢ) was considered to be the HR after complete cardiac autonomic (muscarnic-cholinergic and β₁-adrenergic) receptor blockade. Sympathetic tonus was calculated as HRᵢ – HRₑ, where HRₑ is the heart rate after muscarinic-cholinergic receptor blockade.

Gender differences. Initially, data were analyzed separately for male and female mice within each group. No statistically significant gender differences in numbers of LSG neurons, cardiac NE concentrations, MAP, resting HR, or sympathetic tonus were noted for age/genotype-matched mice (P > 0.05). Hence, data for males and females were pooled for analyses.

Statistical analysis. Mice were grouped according to age and genotype. Data were analyzed using a SigmaStat statistical program. Heart/body weight ratios were transformed by an arcsine square root transformation prior to ANOVA. A square root transformation was performed on neuron counts in the LSG prior to analyses. Differences indicated by ANOVA were elucidated by post hoc tests. Although transformed data were analyzed, data are reported as untransformed values. NE concentrations and sympathetic tonus were compared between corresponding data cells grouped by age and genotype using an unpaired Student's t-test. Alpha levels of 0.05 were used to determine statistical significance in all tests.

RESULTS

Heart/body weight ratios in NT3 +/+ and −/− mice. Weights of +/+ and −/− mice were equivalent at birth, weaning, and adulthood. NT3 +/+ mice weighed 20% less than the other two genotypes at birth. However, hearts of −/− mice were proportionately smaller; thus heart/body weight ratios were equivalent among +/+ and −/− and −/− mice at birth. Likewise, heart/body weight ratios between +/+ and −/− mice were equivalent at weaning and adulthood. Heart/body weight ratios ranged from 5.4–6.2 × 10⁻³ for all groups of mice.

Neuron deficits in the stellate ganglion of NT3 +/+ and −/− mice. The majority of postganglionic cardiac sympathetic neurons originate in the stellate ganglion in the rat (19). Therefore, we examined the LSG (Fig. 2A), to determine whether inactivation of one copy of the NT3 gene resulted in a reduction in the number of cardiac sympathetic neurons. At birth, −/− mice had significantly fewer (P < 0.05) neurons than did +/− mice, which in turn had fewer (P = 0.06) neurons than +/+ mice (Fig. 3A). In addition, the neuron deficit in +/− mice was 22% or approximately one-half that of −/− mice (46%) at birth. Neuron deficits in +/− mice persisted postnatally. The reduction in neuron number in +/− mice, relative to +/+ mice, at P21 and P60 approximated that at birth (25% and 20%, respectively). Neuron number decreased by relatively the same degree in both +/+ and −/− mice between P0 and P21. No significant decreases in neuron number occurred between P21 and P60 in the LSG of either +/+ or −/− mice.

To determine whether increased sympathetic neuron death was occurring in +/+ and −/− mice relative to +/+ mice on P0, we examined neuronal apoptosis in the LSG by a TUNEL assay and anti-TH immunocytochemistry. Despite the observed deficits in sympathetic neurons in −/− and +/+ mice at birth, few (1-3 cells/section) TUNEL /TH⁺ neurons were present in the LSG irrespective of genotype on P0 (Fig. 2, B and C). Collectively, these data suggest that most of the loss of sympathetic neurons occurs prior to birth in mice.

Decreased anti-TH reactivity in NT3 −/− mice. Deletion of one or more NT3 genes qualitatively affected the intensity of anti-TH reactivity in the LSG. Sections of the LSG from +/+ mice bound anti-TH antibodies more intensely than those of −/− mice and less intensely than those of +/+ mice on P0 (Fig. 2, F, D–F). In addition, the LSG of −/− mice bound anti-TH less intensely than the LSG of +/+ mice on P60 (Fig. 2, F, G and H).

Lowered cardiac NE concentrations in NT3 +/+ and −/− mice. The principal neurotransmitter stored in nerve terminals of cardiac sympathetic neurons is NE (15). Thus NE concentrations were measured to provide an index of cardiac sympathetic innervation. Mean cardiac NE concentrations were equivalent among +/+ and −/−, and −/− mice at birth, ranging from 100 to 160 pmg/mg tissue (Fig. 3B). Ventricular NE concentrations increased gradually with age. NE concentrations differed significantly (P < 0.05) between +/+ and −/− mice at weaning and this difference was more pronounced (P < 0.05) in adults (Fig. 3B). Ventricular NE concentrations of adult −/− mice were 37% less than those of +/+ mice (Fig. 3B). Cardiac NE concentrations were higher than those reported for adult mice (15), but this difference reflects a strain difference between Balb/c 129 and C57/B16 mice (Walro, unpublished observations).

The hearts of +/+, −/−, and −/− mice on P0 and +/+ and −/− mice on P21 and P60 were stained with anti-TH antibodies to determine whether low cardiac NE concentrations on P0 and gradually increasing cardiac NE concentrations through P60 were due to the proliferation and growth of cardiac sympathetic nerve endings within the heart. Sympathetic nerve endings were restricted to the epicardium on P0 (Fig. 4, A and B). On P21, sympathetic nerve endings were detected to the greatest extent in the epicardium but were observed coursing with blood vessels in the myocardium in both +/+ and −/− mice (Fig. 4, C and D). By P60, numerous sympathetic nerve endings were visible among myocytes in the myocardium of both +/+ and −/− mice (Fig. 4, E and F). However, no differences in density of these fibers were discernible among mice all
Fig. 2. Left stellate ganglion (LSG) of +/+ (B–D and G), +/− (A, E, and H), and −/− (F) mice in 6-µm paraffin sections on postnatal day 0 (P0; A–F) and postnatal day 60 (P60; G and H). A: the LSG (S) is visible at the level of the second rib (R) and lies adjacent to the subclavian vessel (V). B and C: adjacent sections of LSG of a +/+ mouse. Dark cell (B, arrow) shown to be TUNEL + is also TH + (C, arrow) and is a sympathetic neuron undergoing apoptosis. D–H: anti-TH stained sections of +/+ (D and G), +/− (E and H) and −/− (F) mice on P0 (D–F) and P60 (G and H). Note the difference in staining intensity in the LSG of +/+ mice relative to +/− and −/− mice on P0 and +/+ mice on P60. Scale bars = 250 µm in A and D–H and 40 µm in B and C. TUNEL, terminal transferase-mediated dUTP-biotin nick end labeling; TH, tyrosine hydroxylase. N/A, not available due to premature mortality.

Fig. 3. A: counts of neurons in LSG of NT3 +/+ , +/−, and −/− mice. Note that +/− and −/− mice have fewer sympathetic neurons than age-matched +/+ littermates. Also note that mice on P21 and P60 have fewer neurons than do mice having the same genotype on P0. aDifferent from +/+ mice at P = 0.06. bDifferent from +/− mice at P < 0.05. cDifferent from +/+ mice at P < 0.05. B: cardiac norepinephrine (NE) concentrations in NT3 +/+ , +/−, and −/− mice. Note that NE concentrations are equivalent among +/+ , +/−, and −/− mice on P0. NE concentrations are significantly less in +/− mice than in +/+ mice on P21, and this difference increases with age. aDifferent from +/+ at P < 0.05. bDifferent from newborn at P < 0.05. cDifferent from weanling at P < 0.05. Sample sizes are shown in parentheses. Sample sizes for P0 +/+ , +/−, and −/− mice are 10, 20, and 5, respectively.
Functional cardiac deficits in adult NT3 \(1/2\) mice. Baseline MAP (122 ± 4 vs. 122 ± 6 mmHg) did not differ (\(P > 0.05\)) between \(1/1\) and \(1/2\) mice (Fig. 5A). However, the resting HR of \(1/2\) mice (464 ± 31 beats/min) was significantly lower (\(P < 0.05\)) than that of \(1/1\) mice (557 ± 25 beats/min) (Fig. 5B). Sympathetic tonus \((HR_m - HR_r)\) of adult \(1/1\) mice was nearly twice that of adult \(1/2\) mice (\(P < 0.001\)). Sympathetic tonus of \(1/2\) mice was 125 ± 11 compared with 244 ± 25 beats/min in \(1/1\) mice (Fig. 5C).

**DISCUSSION**

Previous studies of NT3 mutant mice have focused primarily on neural deficits resulting from the deletion of both copies of the NT3 gene (8, 9). A major finding of the present study is that heterozygous mutant \((1/2)\) mice, which lack a single active copy of the NT3 gene, exhibit not only deficits in numbers of cardiac sympathetic neurons but also deficits in neurotransmitter concentrations and sympathetic tonus.

Role of NT3 in the survival of cardiac sympathetic neurons. The importance of NT3 for the survival of sympathetic neurons is well documented. The superior cervical ganglion (SCG) of newborn mice lacking the NT3 gene contains approximately one-half the number of neurons as the SCG from wild-type mice (8, 9). We observed a 46% deficit in the LSG of newborn mice lacking both copies of the NT3 gene and a 22% deficit in mice lacking one copy of the NT3 gene; thus paravertebral sympathetic ganglia other than the SCG are dependent on NT3 for survival prior to birth. These data differ from that of Brennan et al. (2), who found no difference in numbers of SG neurons in \(1/2\) and \(1/1\) NT3 mutant mice.

Excessive cell death due to apoptosis occurs prior to birth in the SCG of NT3 \(2/2\) mice, although data regarding the specific days during gestation when cell death occurs are conflicting (6, 28). The rates of apoptosis in the SCG of NT3 \(2/2\) mice and \(1/2\) mice are equivalent at birth (9). Consequently, the decrease in LSG cell number observed in \(1/2\) and \(-/2\) mice on P0 likely results from excessive cell death prior to birth. We observed few TUNEL \(+/\)TH \(+\) cells in the LSG of \(+/+,\) \(+/-,\) and \(-/-\) mice on P0, which indicates that the rate of apoptosis may be declining postnatally. No qualitative difference in the number of TUNEL \(+/\)TH \(+\) LSG cells among \(+/+,\) \(+/-,\) and \(-/-\) mice on P0 and stable cell numbers in \(+/-\) mice relative to \(+/+\) mice through P60 indicate that lowered levels of NT3 do not lead to a significant increase in postnatal apoptosis of LSG neurons.
and +/+ mice increased gradually with age. TH\(^+\) sympathetic endings were present in the epicardium of +/+ , +/- , and +/- mice on P0 but were not observed in the myocardium of +/- or +/- mice until P60. Collectively, these data reflect the immaturity of sympathetic innervation of the heart at birth and are consistent with data obtained previously in mammals (15, 23, 25). Sympathetic neurons first contact target organs \~9 days before birth in rodents (16, 22), but the sympathetic innervation of the heart may not mature until 60 days after birth (25). Ursell et al. (25) noted that the sympathetic innervation of the dog heart was restricted to the epicardium in mid-late gestational pups and that sympathetic neurons penetrated the myo- and endocardia and matured 2 mo after birth. Thus, although sympathetic neurons reach the mouse heart early in development, the low cardiac NE concentrations in all genotypes of NT3 mice at birth suggest that the penetration and ramification of sympathetic neurons into the myo- and endocardia are postnatal events.

The qualitative density of TH\(^+\) nerve fibers was equivalent in the epicardium of all three genotypes at birth. Similarly, El Shamy et al. (6) observed no qualitative difference in innervation in the hearts of NT3 +/- and +/- mice after TH immunocytochemical staining on P7 (6). In the present study, location and density of TH\(^+\) nerve fibers within the heart were similar in NT3 +/- and +/- mice through P60. However, +/- mice exhibited lower cardiac NE concentrations than did +/- mice at P21, and this deficit became more severe by P60. The increasing disparity in cardiac NE concentrations was not paralleled by a proportionate loss of neurons in the LSG or by a loss of nerve terminals in the heart, thus cell death could not have accounted for the lower cardiac NE concentrations. In the absence of a proportional loss of neurons, reduced synthesis of NE is the most plausible source for the increasing deficits in cardiac NE concentrations in +/- mice relative to +/- mice.

Gene dosage effect of NT3. Deletion of the NT3 gene may affect the size of sympathetic neuron populations in a dose-dependent fashion. The 22% deficit in sympathetic neurons present in NT3 +/- mice at birth was approximately one-half the deficit (46%) observed in NT3 +/- mice. Indirect evidence suggests that the number of cellular copies of NT3 and NGF genes regulates the size of sensory or sympathetic neuron populations. Ernfors et al. (8) noted a 50% reduction in muscle spindles, which exist in a 1:1 ratio with the group Ia afferent and fusimotor neurons that innervate spindles, of NT3 +/- mice. Likewise, deletion of a single copy of the NGF gene results in a 50% reduction in sympathetic neurons (2), and 13–24% fewer calcitonin gene-related peptide (CGRP)-reactive neurons are present in dorsal root ganglion (DRG) L4–5 of NGF +/- mice (4). Although haplo-insufficiency, or deficits in neurotrophin levels of target organs resulting from inactivation of one copy of a gene, has not been documented in NT3 +/- mice, brain-derived neurotrophic factor (BDNF) +/- mice have been shown to have reduced tissue levels of BDNF by ELISA (1). The

Effect of NT3 on cardiac NE concentrations. Cardiac NE concentrations of all three genotypes of mice were low at birth (10–13% of adults), and those of NT3 +/-
deletion of one or more BDNF genes affects the survival of neurons in vestibular and nodose ganglia in a dose-dependent manner. The number of neurons in nodose/petrosal and vestibular ganglia of BDNF +/- mice is intermediate between those of BDNF +/- and +/- mice (1). Haplo-insufficiency may affect the phenotype of sympathetic neurons in addition to affecting their survival. A reduced synthesis of NE in NT3 sympathetic neurons in addition to affecting their survival. In these animals, Ganglion blockade, which blocks both parasympathetic and sympathetic outflow, results in small but nonsignificant decreases in resting HR in mice (21). These data demonstrate that sympathetic drive predominates in maintaining resting HR in the mouse (14, 21). Furthermore, sympathetic drive mediates increases in HR, cardiac output, and AP during exercise or other stressors. The HR of a mouse can increase to ~800 beats/min (21). The mechanisms mediating the increase in HR are vasoconstriction and an increased activity of the sympathetic nervous system (21). Given the important role that the sympathetic nervous system plays in the regulation of cardiac inotropy and chronotropy, NT3 +/- mice may have a reduced ability to respond to exercise or other stressors due to the reduced chronotropic and inotropic reserve. These important questions merit further investigation.

Sympathetic neurons regulate HR mainly through β2-adrenergic receptors (21). However, other receptors also mediate sympathetically induced chronotropic effects. Specifically, α1-adrenergic receptors (20) and the receptors for neuropeptide Y (10) have direct chronotropic coupling in isolated atria. These data may explain the failure to find lower resting HRs in β2-adrenergic receptor knockout mice (21). Importantly, the HRs of β2-adrenergic receptor knockout mice were significantly lower than wild-type mice after blocking cardiac muscarinic receptors. Blockade of parasympathetic outflow reduced HR variability to the point where differences in resting HR could be observed. These data support the notion that parasympathetic outflow has a profound influence on resting HR in the mouse (21).

NT3 +/- mice as a model for cardiac dysautonomias. NT3 +/- mice die within a few weeks of birth due to adverse pleiotropic effects on the outflow vessels of the heart or a general failure to thrive (5, 8, 9). Consequently, researchers have been unable to study how neuron deficits in these mice affect organ function in postnatal mice. Although inactivation of a single copy of the NT3 gene results in significant deficits in the sympathetic nervous system, NT3 +/- mice survive into adulthood. Individuals afflicted with dysautonomias, a broad category of disorders affecting the autonomic nervous system of humans, also survive to adulthood. When the sympathetic nervous system is affected, the hallmarks of the disease include catecholamine deficiencies and loss of myocardial nerve terminals (12). Hence, the phenotype of NT3 +/- mice mimics the phenotype of individuals affected by sympathetic dysautonomias in many respects.

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