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Strain-specific patterns of autonomic nervous system activity and heart failure susceptibility in mice

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Shusterman, Vladimir, Irmute Usiene, Chivonne Harrigal, Joon Sup Lee, Toru Kubota, Arthur M. Feldman, and Barry London. Strain-specific patterns of autonomic nervous system activity and heart failure susceptibility in mice. *Am J Physiol Heart Circ Physiol* 282: H2076–H2083, 2002. First published February 14, 2002; 10.1152/ajpheart.00917.2001.—Transgenic mice are widely used to study cardiac function, but strain-dependent differences in autonomic nervous system activity (ANSA) have not been explored. We compared 1) short-term pharmacological responses of cardiac rhythm in FVB vs. C57Black6/SV129 wild-type mice and 2) long-term physiological dynamics of cardiac rhythm and survival in tumor necrosis factor (TNF)- α transgenic mice with heart failure (TNF- α mice) on defined backgrounds. Ambulatory telemetry electrocardiographic recordings and response to saline, adrenergic, and cholinergic agents were examined in FVB and C57Black6/SV129 mice. In FVB mice, baseline heart rate (HR) was higher and did not change after injection of isoproterenol or atropine but decreased with propranolol. In C57Black6/SV129 mice, HR did not change with propranolol but increased with isoproterenol or atropine. Mean HR, but not indexes of HR variability, was an excellent predictor of response to autonomic agents. The proportion of surviving animals was higher in TNF- α mice on an FVB background than on a mixed FVB/C57Black6 background. The homeostatic states of ANSA are strain specific, which can explain the interstrain differences in mean HR, pharmacological responses, and survival of animals with congestive heart failure. Strain-specific differences should be considered in selecting the strains of mice used for transgenic and gene targeting experiments.

electrophysiology; transgenic models; heart rate

RECENT ADVANCES in genetic engineering have provided the tools for reproducing cardiac pathology in biological models. For example, mouse models have been developed to investigate the pathogenesis of heart failure by overexpressing the pleiotropic peptide tumor necrosis factor (TNF)- α (TNF- α mice) (19). Although these mouse models provide robust platforms for elucidating the pathology of heart muscle disease, the unique physiology of the mouse heart may abrogate the ability to apply measures that are standard in larger animals

(17). Furthermore, little attention has been given to strain-specific physiological differences that might underlie or modify any phenotypic changes in electrophysiological characteristics elicited by transgenic overexpression or gene targeting.

An example of this conundrum is seen when attempting to assess autonomic nervous system activity (ANSA) in mice. Although of critical importance in the pathology of heart muscle disease, ANSA is difficult to monitor effectively in experimental animals because of 1) the complex relationships between cardiac rhythm dynamics and ANSA, 2) the varied effects of hormone status, respiratory rate, body temperature, and activity level on ANSA, 3) the inability of a controlled environment to reproduce the natural pattern of ANSA during regular daily activities, and 4) the presence of largely random variations in external and internal modifiers of ANSA (1, 8, 26, 29). Indeed, attempts to measure ANSA in mouse models have resulted in markedly different findings both in baseline heart rate (HR) and in responses to autonomic agents (7, 12, 14, 16, 17, 21, 30, 31).

We hypothesized that a significant portion of the differences between various wild-type and transgenic mouse models might be attributable to intrinsic variability in autonomic activity in different mouse strains. To test this hypothesis, we measured HR variability and pharmacological responses in three different experimental settings: 1) wild-type FVB mice, a common strain used in transgenic overexpressors; 2) C57Black6/SV129 mice, the most common strain found in gene-targeted mice; and 3) TNF- α mice (on a FVB background) that develop a dilated cardiomyopathy by 12 wk of age. We found that the homeostatic states of ANSA are strain specific and that this can explain the interstrain differences in mean HR, the responses to pharmacological agents, and the blunted HR response in the TNF- α mouse. It may also lead to the marked strain-dependent differences in mortality.

METHODS

Ambulatory telemetry recordings were examined in 10 FVB mice (age 3–9 mo; 3 female, 7 male), 9 C57Black6/

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SV129 mice (age 3–15 mo; 5 female, 4 male), and 13 TNF- α transgenic mice engineered on an FVB background (age 3–9 mo; 6 female, 7 male). FVB mice were used because they are the most common strain used for overexpression transgenics, whereas C57Black6/SV129 hybrids were used because most gene-targeted (knockout) mice are engineered in SV129 stem cells and backcrossed into C57Black6. In this study, C57Black6/SV129 mice were the results of three to five backcrosses of 50% C57Black6/50% SV129 mice into C57Black6 mice and were thus $\geq 90\%$ C57Black6. Radiotelemetry devices (DATA Sciences) were implanted subcutaneously on the backs of the mice. Twenty-four-hour recordings were performed 6 days later with the MacLab recording system, and animals were monitored for the duration of the drug study (2 wk). A subset of mice was monitored for up to 5 mo.

Electrocardiographic data were digitized at 400 Hz and 16-bit resolution and interpolated to 1,600 Hz with cubic spline to enhance the time resolution. QRS complexes were classified with custom software and verified by an experienced Holter technician. The RR intervals between normal QRS complexes were extracted, and a regularly spaced time series was sampled at 16 Hz with a boxcar low-pass filter (3). Gaps in the time series resulting from noise or ectopic beats were filled in with linear splines, which can cause a small reduction in high-frequency power but do not affect other components of the power spectrum (2).

We found that the mean HR in control mice [645 ± 48 beats/min (bpm)] was approximately eight times higher than in humans, assuming an average HR in healthy humans of 80 ± 7 bpm (23). Therefore, the time intervals and time- and frequency-domain variables were adjusted for these high HRs. Time- and frequency-domain analysis of RR intervals was performed over the entire 24-h period. The time course of changes in RR intervals during pharmacological tests was analyzed in 37.5-s intervals and averaged for each consecutive 7.5-min interval. To estimate the pharmacological effects, four consecutive 7.5-min windows before and four consecutive 7.5-min windows after the injection were included in the nonparametric ANOVA. The mean values represent averages over 30 min before and after the injections. The duration of the time intervals for analysis was obtained by dividing the 300- and 3,600-s intervals, respectively, traditionally used in humans by 8.

Time domain analysis. Mean HR, standard deviation of normal RR intervals (SDNN), the square root of the mean of the squared differences between adjacent normal RR intervals (r-MSSD), and the percentage of those differences between adjacent normal RR intervals >6 ms (pNN6) were estimated such that pNN6 was analogous to the percentage of the differences between adjacent normal RR intervals that are >50 ms (pNN50) in humans (4).

Frequency domain analysis. After the mean was subtracted from the time series, power spectral analysis was performed with fast Fourier transform and a Hanning window. Zero padding was applied to increase the outcome frequency resolution, and the resulting power spectrum was corrected for the filtering and windowing (22). The frequency ranges were obtained from the values used for the analysis of human data by multiplying by 8, which is similar to the computations used in previous investigations (Fig. 1; Refs. 12 and 30). Power was integrated in high (HFP; 1.2–3.2 Hz), low (LFP; 0.32–1.2 Hz), and very low (VLFP; 0.0264–0.32 Hz) frequency ranges, and the ratio of low- to high-frequency power (LFP/HFP) was also calculated. We did not use the normalized LFP and HFP, because they provide essentially the same information as the ratio LFP/HFP (8). The ultra-low-frequency component (ULFP; 0–0.0264 Hz) and the 24-h

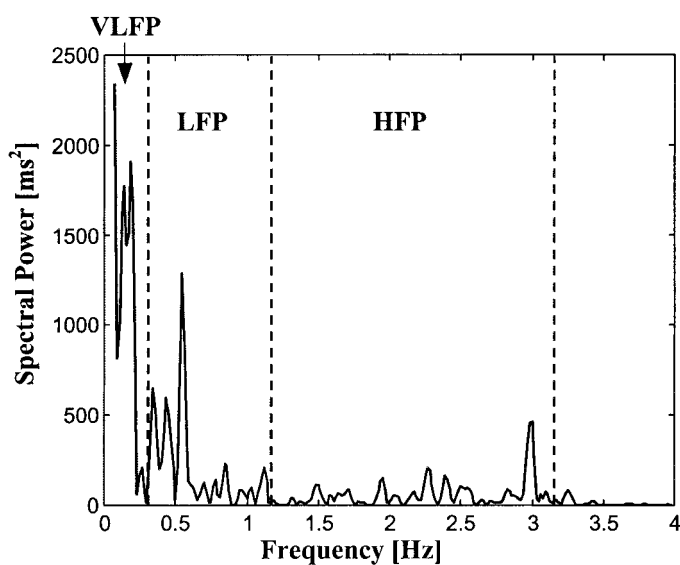


Fig. 1. A representative example of a spectral power distribution in a 37.5-s segment of cardiac cycles obtained from a control mouse. The spectrum has 3 peaks: a respiratory peak at 3 Hz in the high-frequency region (HFP), a peak near 0.5 Hz in the low-frequency region (LFP), and a peak below 0.2 Hz in the very-low-frequency region (VLFP) of the spectrum.

total power (TP; 0–3.2 Hz) were determined over the entire recording for comparison between different strains.

Pharmacological tests. Tests were performed at the same time of day and in the same room; only one test was performed on each given day. The pharmacological tests were performed in the following order: saline, isoproterenol (1 μ g), propranolol (4 mg/kg), atropine (1 mg/kg), carbamyl choline (carbachol; 0.5 mg/kg), methoxamine (6 mg/kg), prazosin (1 mg/kg), and saline. Serial changes in cardiac rhythm were examined during 30 min before and after each intraperitoneal injection. Methoxamine was dissolved in 0.1% DMSO. Injection with 0.1% DMSO alone had effects similar to those of saline (data not shown).

Strain-dependent survival. Male TNF- α mice (X-linked transgene) were mated with FVB or C57Black6 females. Survival of female TNF- α transgene offspring was compared on the FVB vs. 50% FVB/50% C57Black6 background with the Kaplan-Meier cumulative survival curve and Gehan's Wilcoxon test.

Statistical analysis. Comparisons between strains were performed with nonparametric Mann-Whitney *U*-test. Changes in the variables during pharmacological tests were estimated with nonparametric Friedman ANOVA for repeated measurements. Nonparametric Spearman correlations were used to assess the relationship between mean HR and its reaction to pharmacological tests. Results are presented as means \pm SD unless otherwise indicated. Statistical significance was accepted at the level of $P < 0.05$.

RESULTS

Twenty-four-hour differences in strain patterns of cardiac rhythm. FVB mice had faster 24-h mean HRs than C57Black6/SV129 mice (Table 1). SDNN and TP, gross measures of HR variability, and pNN6, a measure of short-term rhythm irregularity, were significantly lower in FVB mice than in C57Black6/SV129 mice.

Short-term pharmacological responses to autonomically active agents in FVB and C57Black6/SV129 strains. Injection of saline caused a stress response manifested by an increase in HR and a decrease in

Table 1. Twenty-four-hour indexes of HR variability in C57Black6/SV129, FVB, and TNF- α mice

	C57Black6/SV129	FVB	TNF- α	P_1	P_2
Total N/N in SR	9/9	10/10	13/9		
HR	609 \pm 22	680 \pm 33	632 \pm 66	0.0002	0.05
TP	1,747 \pm 1,045	777 \pm 538	525 \pm 274	0.02	0.5
ULFP	1,362 \pm 935	592 \pm 541	398 \pm 235	0.04	0.6
VLP	199 \pm 123	90 \pm 44	60 \pm 32	0.06	0.1
LFP	127 \pm 91	56 \pm 37	30 \pm 21	0.1	0.1
HFP	58 \pm 55	38 \pm 27	37 \pm 21	0.4	0.9
LFP/HFP	2.4 \pm 1.0	1.6 \pm 0.3	1.1 \pm 0.6	0.07	0.06
SDNN	9.9 \pm 3.0	6.3 \pm 1.7	5.9 \pm 1.3	0.01	0.7
r-MSSD	4.4 \pm 3.2	17 \pm 28	3.4 \pm 1.3	0.4	0.5
pNN6	3.2 \pm 2.2	1.1 \pm 0.8	1.8 \pm 1.3	0.02	0.2
PVC/h	0.7 \pm 0.5	0.8 \pm 0.9	57 \pm 123	0.7	0.01
PAC/h	1.0 \pm 1.5	1.3 \pm 1.0	18 \pm 26	0.06	0.004
V runs	0	0	36		

Parameter values are means \pm SD for N mice. SR, sinus rhythm; P_1 , significance of the difference between C57Black6/SV129 and FVB mice; P_2 , significance of the difference between FVB and tumor necrosis factor (TNF)- α transgenic mice with heart failure (TNF- α mice) on a pure FVB background; HR, heart rate beats/min; TP, total power; VLP, very low-frequency power; LFP, low-frequency power; HFP, high-frequency power; SDNN, standard deviation of normal RR intervals; r-MSSD, square root of the mean of squared differences between adjacent normal RR intervals; pNN6, % of the differences between adjacent normal RR intervals that are >6 ms; PVC, premature ventricular complexes in beats/h; PAC, premature atrial complexes in beats/h; V runs, ventricular runs that have ≥ 5 consecutive beats of ventricular origin.

SDNN (Figs. 2 and 3A) that peaked at 8–15 min (second 7.5-min window) and lasted ~ 50 min (Table 2). The peak response to other agents injected intraperitoneally was similarly delayed. To control for the changes in the stress response over time, injections of saline were given at the beginning and at the end of the protocol. The differences between the two responses were not statistically significant.

Stimulation of β -adrenergic receptors with isoproterenol did not cause any changes in HR or SDNN in FVB mice (Figs. 2 and 3). However, r-MSSD, an index of short-term HR variations, increased in this group compared with saline (Fig. 3). In C57Black6/SV129 mice, which had lower baseline HRs and higher HR variability, isoproterenol caused an increase in HR, a decrease in SDNN, and no change in r-MSSD.

The effect of parasympathetic blockage with atropine was similar to that of isoproterenol. Atropine did not change HR or SDNN in FVB mice but increased HR and decreased SDNN in C57Black6/SV129 mice. In both groups, atropine reduced the spectral indexes of HR variability, HFP and LFP, and decreased the ratio LFP/HFP. Of note, changes in the short-term indexes of HR variability in C57Black6/SV129 were incoherent: r-MSSD increased, whereas pNN6 declined.

In response to propranolol, HR decreased in FVB mice but did not change in C57Black6/SV129 mice. However, propranolol did prevent the increase in HR in the latter group seen with the injection of saline. In FVB mice, the HR variability did not change, contrasting the pronounced reaction of HR. Although the HRs were unchanged in C57Black6/SV129 mice, SDNN and LFP declined.

Responses to the modifications of α -adrenergic activity were similar in both groups. Stimulation of α_1 -adrenergic receptors with methoxamine decreased HR and increased its total variability, reflected in SDNN. The short-term variability (r-MSSD, pNN6, and HFP)

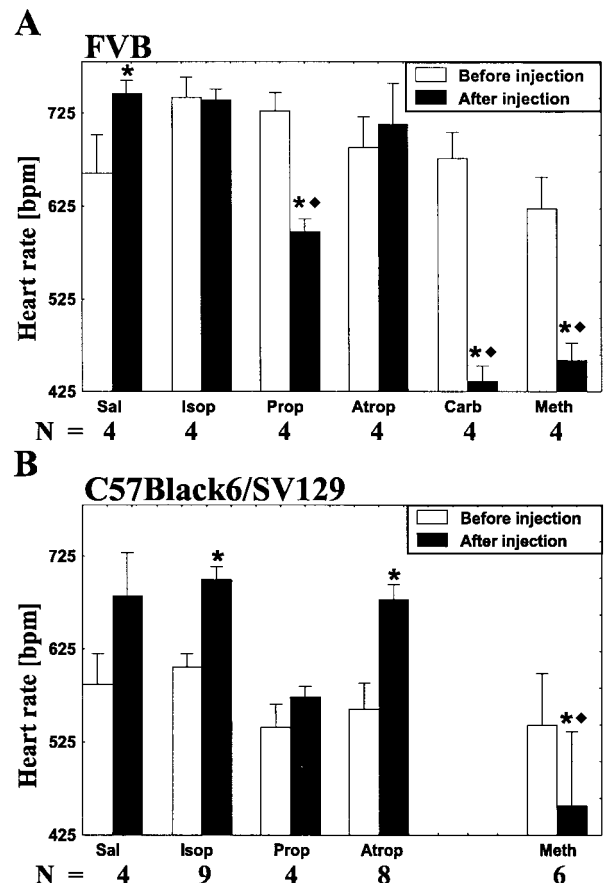


Fig. 2. Changes in heart rate (HR) in response to pharmacological modifications of autonomic nervous system activity in FVB (A) and C57Black6/SV129 (B) mice. The magnitude and direction of the changes are determined by the baseline HR and its dynamic range (see text). Sal, saline; Isop, isoproterenol; Prop, propranolol; Atrop, atropine; Carb, carbachol; Meth, methoxamine. N , no. of animals used for each test. Statistically significant ($P < 0.05$) changes compared with the corresponding baselines (*) and the effect of saline (♦) are indicated. Error bars indicate corresponding SE.

Table 2. Time of occurrence of peak change in HR and SDNN and duration of pharmacological effect from time of injection

Pharmacological Agent	Peak		Duration	
	HR	SDNN	HR	SDNN
Saline	8–15	8–15	45–53	45–53
Isoproterenol	8–15	15–23	45–53	38–60
Atropine	1–8	15–23	>60	>60
Carbachol	8–15	8–15	>60	30–45
Methoxamine	1–8	15–23	>60	>60
Prazosin	1–8	15–23	>60	>60
Propranolol	8–15	15–23	>60	>60

Values are expressed in minutes.

also increased, but the upsurge in LFP was greater, causing a rise in LFP/HFP. The effects of α_1 -adrenergic blockage with prazosin were small and did not reach statistical significance (data not shown).

Long-term physiological dynamics of cardiac rhythm in FVB and TNF- α strains. Four TNF- α mice had continuous atrial arrhythmias and were excluded from HR variability analysis. Mean HRs were somewhat slower in TNF- α mice in sinus rhythm compared with FVB control mice (Table 1).

The frequency of premature ventricular and supraventricular complexes was higher in TNF- α mice compared with FVBs (Table 1). Serial ambulatory telemetry recordings from three TNF- α mice showed progressive decrease in HR, whereas the frequency of ventricular ectopy and its complexity increased in two of the three animals (Table 3). Ventricular runs occurred two times more often after the age of 3 mo than at a younger age. All time (SD, r-MSSD, and pNN6)- and most frequency (TP, VLFP, and LFP)-domain indexes and the ratio LFP/HFP also increased with time in TNF- α mice.

Survival in TNF- α mice with congestive heart failure on different genetic backgrounds. The proportion of surviving animals with heart failure was significantly higher in female TNF- α mice on an FVB background than in female TNF- α mice on an FVB/C57Black6 background. (Fig. 4).

Relationship to prior murine studies of cardiac rhythm. Our data suggested that the response to autonomic agents is strain dependent and correlates with baseline HR. We compared our data with that of nine prior studies (Table 4). In those studies that monitored HR for >1 h, wild-type strains with higher average HRs had greater propranolol-induced HR deceleration ($r = -0.94$, $P = 0.005$; $n = 6$) and smaller atropine-induced HR acceleration ($r = -0.75$, $P = 0.05$; $n = 7$). Mean HR in unrestrained wild-type mice was an excellent identifier of the strain response to β -adrenergic blockage [$r = -1.0$; $n = 5$, excluding the data by Uechi et al. (30)].

DISCUSSION

Main results and comparison with previous studies. A strong relationship was identified between mean HR in the long-term ambulatory recordings and strain-

specific pharmacological responses. Mice of the FVB strain had a high mean HR, large β -blocker-induced HR deceleration, and small atropine-induced acceleration of HR. Mice of the C57Black6/SV129 strain had lower mean HR, small HR response to β -blocker, and large atropine-induced HR increase. This suggests that FVB mice have higher basal sympathetic and lower parasympathetic activity than C57Black6/SV129 mice and that mean HR is an excellent indicator of the strain-specific homeostatic state of ANSA.

The greater contribution of sympathetic activity in the homeostatic state of ANSA in mice compared with larger mammals is well known and has led to the assumption that all strains have similar, sympathetically dominated states of ANSA (17). The strain differences in HR and responses to pharmacological tests remained unexplained. Furthermore, the reasons for divergent cardiac rhythm dynamics in different transgenic mouse models of congestive heart failure were also unclear (19, 30). The interstrain differences in homeostatic ANSA could provide a plausible link between these unexplained observations.

Baseline long-term HR recordings provided an excellent predictor of the magnitude and duration of pharmacological responses. Because short-term (<1 h) measurements are affected by a number of transient external and internal variables that do not reliably represent the homeostatic state of ANSA, the relationship between mean HRs and pharmacological responses was weak. In addition, the short-term measurements are usually performed under conditions that do not reflect the entire spectrum of natural ANSA variations. Therefore, short-term recordings did not expose interstrain differences in HR (7). Long-term ambulatory monitoring in unrestrained animals is desirable for accurate characterization of strain-specific ANSA.

Short-term HR responses to pharmacological tests. The injection of saline induced a stress response that is likely due to endogenous catecholamines, and the duration of this effect was similar to that of isoproterenol injected intraperitoneally (Table 2). Therefore, appropriate controls are required to distinguish pharmacological effects.

The highest baseline and pharmacologically modified HRs were similar in this and previous studies, which may reflect the existence of an upper limit of sinus node automaticity in the mouse (Table 4). Reaching the upper limit may explain blunted HR responses to β -adrenergic stimulation in FVB mice, whose HRs were initially high.

In our study, the responses to β -adrenergic stimulation and parasympathetic blockage were similar (Fig. 2). Both isoproterenol and atropine increased the HRs in C57Black6/SV129 mice but had no effect in FVB mice. Thus the homeostatic state of ANSA could be shifted in a similar way by modifying either sympathetic or parasympathetic activity, which does not support the traditional point of view of negligible parasympathetic effects in all mice (17). Our results are not

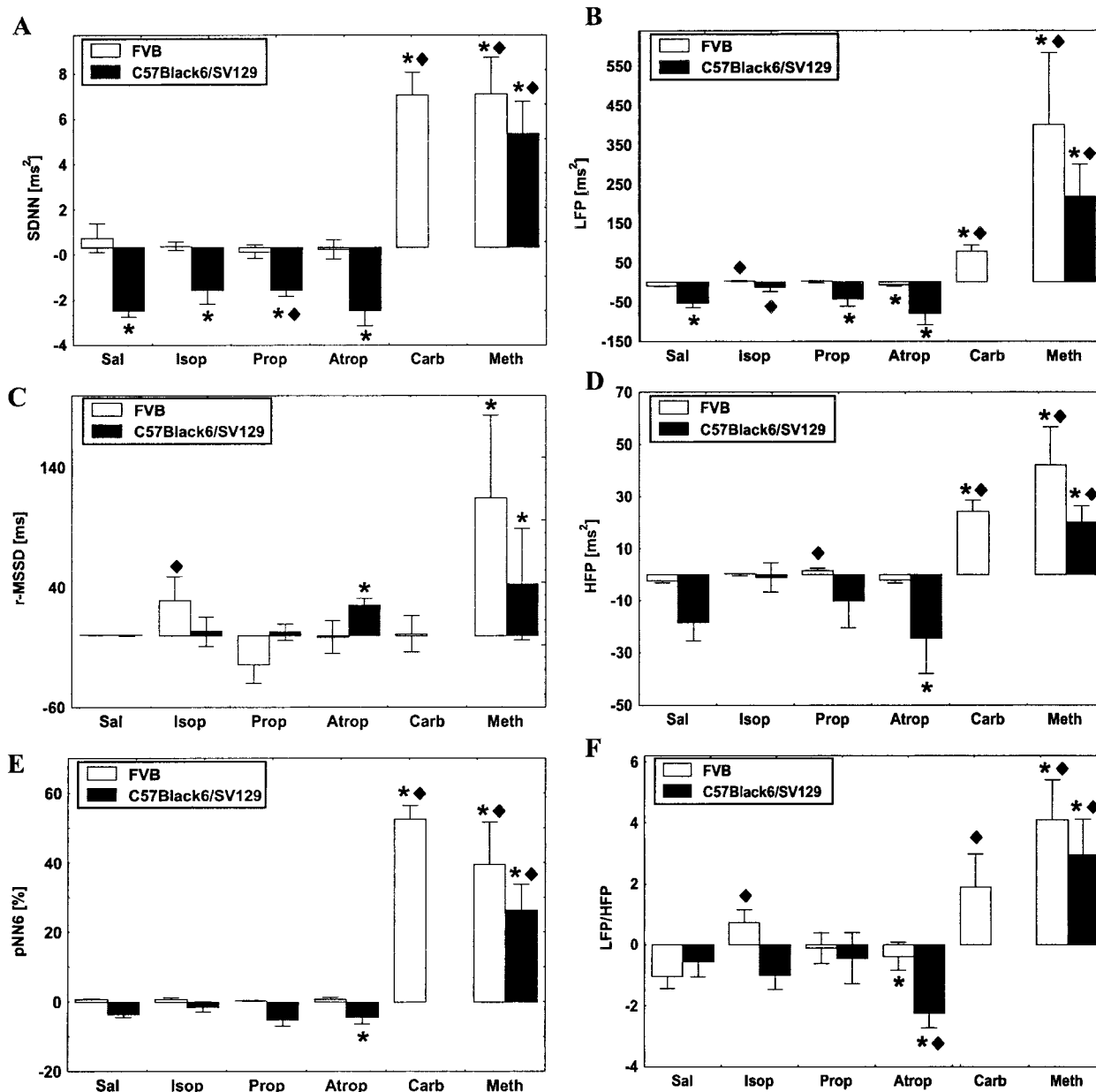


Fig. 3. Changes in the time-domain (A, C, E) and frequency-domain (B, D, F) indexes of HR variability in FVB and C57Black6/SV129 mice in response to pharmacological modifications of autonomic nervous system activity. The association between the studied indexes and the modifications of β -adrenergic and parasympathetic activity is weak; the direction of the changes depends on the corresponding baseline values. Modifications of α -adrenergic activity, however, elicit concordant changes in the studied indexes in both groups. SDNN, standard deviation of normal RR intervals; r-MSSD, square root of the mean of the squared differences between adjacent normal RR intervals; pNNE, % of differences between adjacent normal RR intervals that are >6 ms; LFP/HFP, ratio of low- to high-frequency power. Symbols and no. of experiments as in Fig. 2.

inconsistent with data previously reported by other groups (8, 14, 30).

Specificity of cardiac rhythm indexes for analysis of changes in ANSA. Several fundamental features of the HR variability analysis may affect its representation of ANSA. First, the association between the HR variability indexes and specific changes in ANSA is not strong even in the controlled experiments (8, 13). Second, in ambulatory conditions, this association is further diminished by varying respiration and other physiologi-

cal and external transients (26). Third, HR variability responses depend on individual baseline values, leading to divergent effects of the same stimulus in different subjects (18). They also depend on the intensity of the stimulation and could have different dose-response curves for different pharmacological agents (13, 27).

We sought to determine the indexes of HR variability whose changes during ANSA modifications demonstrated concordant trends in both FVB and C57Black6/SV129 mice and did not depend on baseline values.

Table 3. Serial changes in HR variability indexes in three TNF- α mice on a pure FVB background

	M4		M18		M19		
	5.6 mo	8.6 mo	2.53 mo	4.2 mo	6.6 mo	2.53 mo	3.3 mo
HR, beats/min	606	564	528	496	376	633	397
TP	564	1,043	684	758	869	1,899	1,804
VLP	88	241	76	157	353	221	263
LFP	29	74	27	222	252	43	56
HFP	9.0	13	6.5	46	43	15	12
LFP/HFP	4.2	5.7	4.1	5.3	6.1	3.1	8.8
SDNN	6.5	8.9	6.4	7.9	9.2	12	18
r-MSSD	20	48	6.0	22	1,077	18	38
pNN6	2.7	7.5	3.0	43	108	36	65
PVC/h	2.5	355	52.1	21.7	54.8	10	309
V runs	0	3	6	0	8	1	18

Values in months indicate age of mouse at time of measurement.

Most previous studies observed a weak correspondence between the changes in HR variability and specific ANSA modifications (14, 15, 17, 30). We also found that the association between the indexes of HR variability and modifications of β -adrenergic and parasympathetic activity was weak and affected by the corresponding baseline values. In FVB mice with high baseline HR and low HR variability, the effects of atropine and propranolol on total HR variability were small, whereas in C57Black6/SV129 mice with lower baseline HR and greater HR variability, the effects of these agents were more pronounced (Fig. 3A). Thus caution is required for interpretation of HR variability in terms of specific changes in ANSA. An approximate adaptation of the frequency ranges derived for larger mammals to those in mice is possible (17). However, in uncontrolled ambulatory conditions, gross measures of HR variability and individually tailored pattern recognition techniques may provide more reliable indication of changes in ANSA than spectral methods (16, 26).

The oscillatory nature of the HR variability characteristics requires HR to be stable during the investigated period. Because the peak pharmacological effect of HR usually occurs 8–15 min after intraperitoneal

injection, the HR variability changes are further delayed (Table 2).

Modifications of α -adrenergic tone elicited concordant changes in FVB and C57Black6/SV129 mice. In particular, stimulation of α -adrenergic receptors caused predominant increase in the low-frequency oscillations manifested by an increase in the ratio LFP/HFP. This confirms previous observations that α -adrenergic control of vasomotor oscillations in mice is confined to frequencies below 1 Hz (15, 17).

Long-term dynamics of cardiac rhythm and survival in transgenic mice with heart failure. Recordings from conscious, unrestrained FVB mice showed fast HRs and pronounced β -blocker responses, which suggests that intrinsic sympathetic activity is high. HR in young TNF- α mice was similar to that in FVB controls but tended to decrease with age. The lack of increased HR despite the presence of heart failure in TNF- α mice may depend in part on the baseline high sympathetic activity in the FVB background. Thus the choice of FVB as a background strain for studying the ANSA phenotype and changes in cardiac rhythm during the development of heart failure might not be optimal.

The slowing and increased irregularity of cardiac rhythm that was seen in older TNF- α mice might indicate sinus node dysfunction and the development of supraventricular arrhythmias. This is consistent with our findings of atrial flutter with variable block in many of these animals with optical mapping (data not shown; Ref. 20). In human studies, atrial arrhythmias, blunted responses of HR to pharmacological and physiological sympathetic stimulation, and enhanced short-term HR irregularity have been found in patients with advanced heart failure and poor prognosis (5, 6, 24).

A transgenic mouse model that overexpressed $G_{s\alpha}$ in the heart was engineered in a wild-type strain with lower mean HR and, presumably, lower sympathetic activity (30). Here, the heart failure syndrome was associated with an increase in HR (30). Differences in the background strains might be responsible for the divergence of HR dynamics between this and the TNF- α model of heart failure. In addition, the $G_{s\alpha}$ model directly manipulates sympathetic response and may not be generalizable to other mouse models. In

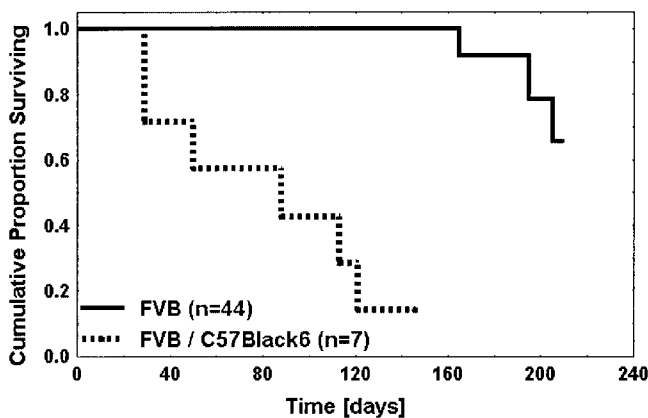


Fig. 4. Kaplan-Meier cumulative survival plot in the 2 strains of tumor necrosis factor (TNF)- α transgenic mice with heart failure (TNF- α mice) with different genetic backgrounds. The cumulative proportion of surviving mice with an FVB background was significantly higher than that with a 50% FVB/50% C57Black6 background ($P < 10^{-5}$).

Table 4. Mean HR and responses to β -adrenergic and parasympathetic blockages in different strains

Study	Time Interval	Strain	WT			TG			
			Resting HR, beats/min	Change in HR, beats/min %		Resting HR, beats/min	Change in HR, beats/min %		
				β -Adrenergic Blockage	Atropine		β -Adrenergic Blockage	Atropine	
<i>Short term</i>									
Mansier et al. (21)	3 min	BL6/DBA	500	-118/-24		β_1 -Adrenoceptor overexpression	450	+23/+5 (NS)	
Desai et al. (7)	<30 min	6-Strain average*	503	-126/-25	+30/+6				
Ishii et al. (14)	3 min	ICR-Jcl	576	-77/-13	+116/+20				
Jumrussirikul et al. (16)	5-10 min	129 and BL6	650	-84/-13	+49/+8	nNOS knockout	711	-84/-12	-2/-0.3
<i>Intermediate term</i>									
Wickman et al. (31)	3 h	BL6/129	647	Decrease	+27/+4 (NS)	GIRK4 knockout	647	Decrease	+14/+2 (NS)
Just et al. (17)	1 h	BL6	662	-119/-18	-61/-9 (NS)				
Gehrmann et al. (12)	1 h	BL6	724	-220/-30	-25/-3 (NS)				
<i>Long term</i>									
Uechi et al. (30)	48 h	BL6	568	-85/-15 [†]	+94/+17 [†]	$G_{s\alpha}$ overexpression	696	-142 /-20	+18/+3
Present study	24 h	BL6/129	606	+14/+2 (NS)	+115/+19				
Janssen et al. (15)	48 h	Swiss	670	-124/-20	+20/+3 (NS)				
Present study	24 h	FVB	680	-168/-25	+19/+3 (NS)				

WT, wild-type mice; TG, transgenic mice; BL6, C57Black6; 129, SV129; nNOS, neuronal nitric oxide synthase; NS, not statistically significant ($P > 0.05$) vs. baseline. *Only mean values for the 6 studied strains (C3H/HeNcrLBR, FVB/NcrLBR, C57Black6, Swiss Webster, SV129, CD-1) were published. [†]Tests performed in restricted animals.

both TNF- α and $G_{s\alpha}$ mice, LFP/HFP was decreased because of an increased proportion of the high-frequency elements in the cardiac rhythm dynamics. This pattern differed from the predominant increase in the low-frequency components and LFP/HFP in response to α -adrenergic stimulation (Fig. 3). The increased proportion of the high-frequency components in heart failure could be explained by reduction of LFP and increased contribution of nonneural mechanisms to HFP, similar to what has been observed in humans (9, 25).

The proportion of surviving animals was significantly greater in TNF- α mice with heart failure on an FVB background than in mice on an FVB/C57Black6 background. Although higher vagal activity is generally viewed as cardioprotective, in heart failure, the shift of ANSA toward increased sympathetic tone is important for providing inotropic support to the failing myocardium and maintaining adequate pressure and perfusion to peripheral tissues (10, 11). Thus it is possible that the TNF- α mice with heart failure on an FVB/C57Black6 background, who presumably had a lower sympathetic and a higher parasympathetic activity, lacked the compensatory effects of heightened sympathetic tone. Although other explanations may exist, it is possible that the higher intrinsic sympathetic activity in TNF- α mice on the FVB background might be associated with beneficial compensatory effects in animals with impaired cardiac function.

Limitations. In our study, the dose-response curves were not examined for the pharmacological modifications of ANSA. Different strains could have distinctive

dose-response curves that would amplify but not eliminate the strain differences in cardiac cycle dynamics. Thus the main conclusions of this study regarding the strain-specific homeostatic states of ANSA would not be affected.

We compared mean HR and response to pharmacological agents in FVB mice vs. C57Black6/SV129 mice. Because of the back crossing, >90% of the genes in the mixed C57Black6/SV129 mice should have been derived from the C57Black6 strain. However, we cannot exclude a contribution of SV129 genes to the lower sympathetic activity in the mixed strain.

A direct comparison of pharmacological effects on the TNF- α and FVB mice was not conducted in the unrestrained conscious state. In anesthetized animals with pronounced bradycardia, isoproterenol increases HR to a similar extent in TNF- α and FVB mice (19). Serial changes in pharmacological responses that accompany development of the heart failure in the TNF- α mice require further study. Comparison of HR and pharmacological responses in TNF- α mice on an FVB background and on an FVB/C57Black6 background might provide more evidence regarding the differences in ANSA in the two strains.

The small sample size of the examined strains could have affected the results of statistical comparisons. To minimize the effects of the sample size and the distribution differences, nonparametric tests were used for statistical analysis.

The strain differences in blood pressure and in intrinsic HR after combined blockage of β -adrenergic and



muscarinic receptors were not examined in this study. These experiments, although not crucially important for the goal of this investigation, could provide additional information about homeostatic states of ANSA in each strain. The data regarding HR and pharmacological responses in TNF- α mice on an FVB/C57Black6 background was unavailable at the time of this study, precluding direct comparison of ANSA in TNF- α mice on an FVB/C57Black6 and on a pure FVB background.

In conclusion, interstrain differences in ANSA are important when studying transgenic models. The homeostatic states of ANSA are strain specific, leading to differences in HR, responses to pharmacological agents, and pathophysiological changes in disease models. Strain-specific differences should be considered in selecting the strains of mice used for transgenic and gene targeting experiments. Genetic mouse models in different backgrounds might be useful for understanding genetic determinants of cardiac rhythm and ANSA in humans (28).

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