

Chronic fluoxetine reduces autonomic control of cardiac rhythms in rats with congestive heart failure

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Henze M, Tiniakov R, Samarel A, Holmes E, Scrogin K. Chronic fluoxetine reduces autonomic control of cardiac rhythms in rats with congestive heart failure. *Am J Physiol Heart Circ Physiol* 304: H444–H454, 2013. First published December 7, 2012; doi:10.1152/ajpheart.00763.2012.—Up to 40% of patients with heart failure develop depression, and depression is an independent risk factor for cardiovascular mortality in this patient population. Consequently, increasing numbers of patients with heart failure are treated with antidepressants. Selective serotonin reuptake inhibitors are typically the antidepressant of choice since this drug class has limited cardiovascular toxicity. However, little is known about the effects of selective serotonin reuptake inhibitors on autonomic cardiac regulation in congestive heart failure (CHF). Here, indexes of cardiac autonomic control were evaluated before and during chronic fluoxetine (FLX) treatment (20 mg·kg⁻¹·day⁻¹, 5 wk) in rats that developed CHF after coronary artery ligation. FLX reduced the low-frequency (LF) component of heart rate variability (HRV; $P < 0.01$) as well as the sympathetic contribution to LF HRV ($P < 0.01$) in both CHF and sham-operated rats. Both FLX and CHF reduced high-frequency HRV ($P < 0.01$). Spontaneous baroreflex gain was decreased in CHF rats 8 wk after ligation ($P < 0.01$). Cross-spectral coherence between the interbeat interval and mean arterial pressure was reduced in the LF domain 3 wk after ligation in CHF rats ($P < 0.01$) and was further reduced after chronic FLX treatment ($P < 0.01$). Plasma catecholamines and LF blood pressure variability were not affected by FLX. Chronotropic responses to both efferent vagal nerve stimulation and isoproterenol administration were reduced in CHF rats and by FLX ($P < 0.01$), whereas inotropic responses to isoproterenol were reduced only in CHF rats ($P < 0.01$). These data indicate that chronic FLX reduces the responsiveness to autonomic output controlling cardiac rhythm and may further compromise autonomic regulation of cardiac function in CHF.

heart rate variability; selective serotonin reuptake inhibitor

IT IS NOW WELL RECOGNIZED that patients who experience a myocardial infarction and subsequently develop congestive heart failure (CHF) have a high risk (up to 40%) of developing mood and anxiety disorders (13). Moreover, a diagnosis of depression is associated with increased morbidity and mortality in CHF patients. Individuals diagnosed with depression or anxiety disorders without underlying cardiovascular disease at the time of diagnosis also have a greater risk of dying of cardiovascular disease than do individuals without depression (54). As a consequence, increasing numbers of cardiovascular patients are being treated with antidepressant drugs. The most common antidepressant drugs used for the treatment of cardiovascular patients are the selective serotonin reuptake inhibitors

(SSRIs) due to their relatively limited cardiovascular toxicity (43). However, little is known about how SSRIs influence cardiac autonomic control in patients with CHF.

Both CHF patients and patients with anxiety disorders show evidence of altered autonomic control of cardiac rhythm, which is itself a risk factor for cardiovascular morbidity and mortality (37). Autonomic dysfunction is manifested, in part, as a decrease in heart rate variability (HRV), a phenomenon that is predictive of arrhythmia and sudden cardiac death in CHF patients (26). Low-frequency (LF) oscillations in heart rate (HR) are mediated by baroreflex-dependent sympathetic and parasympathetic modulation of sinoatrial node cell depolarization (1, 3). The amplitude of the oscillations (and thus absolute power) in the LF domain depends, in part, on the underlying sympathetic tone that is modulated by the baroreflex. High-frequency (HF) oscillations are due primarily to respiratory-dependent effects on the parasympathetic control of HR and, to a lesser extent, to stretch of sinoatrial node cells during ventilation (34, 39). In heart failure, loss of HRV is indicative of decreased cardiac vagal drive and an inability of the parasympathetic system to properly buffer elevated cardiac sympathetic drive, which contributes to ventricular arrhythmogenesis in CHF (42). In dogs subjected to pacing-induced heart failure, increased sympathetic drive to the heart is reflected by increased HRV in the LF domain in the early stages of the disease (36). However, with disease progression, LF oscillations in HR diminish, likely due to reduced sensitivity of cardiac β -receptors (4).

An effect size meta-analysis (19) revealed that depressed individuals treated with SSRIs showed a slight improvement in HRV when variability was assessed over a short 5-min time-frame (49). In contrast, studies (24, 28) examining the effect of SSRIs when variability was assessed over 24 h showed contradictory results with evidence of an increase, decrease, or no change in HRV. While SSRIs have been shown to improve mood and perceived quality of life after myocardial infarction, the effect of SSRIs on cardiovascular outcomes remains controversial (19, 48). SSRI treatment has been shown to reduce cardiovascular-related morbidity and mortality in depressed patients after myocardial infarction (45). Depressed patients treated with SSRIs have also been shown to have an accelerated recovery of HRV after myocardial infarction compared with placebo-treated patients (35). In contrast, some patient populations show decreased HRV with chronic SSRI treatment (8, 31). Results from a more recent small-scale study (33) now suggest that the reduced HRV found in depressed patients may be related to antidepressant medication. Assessments of the effect of chronic SSRI treatment on HRV in patients with CHF is complicated by patient use of additional drugs that influence

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autonomic control of HR, including β -blockers and angiotensin-converting enzyme inhibitors.

Analyses of cardiac autonomic control in humans are often-times limited to measures of HRV and baroreflex control. But these measures alone do not provide evidence of whether altered function derives from the central nervous system or changes in receptor function. Therefore, we investigated the effects of chronic SSRI treatment with fluoxetine (FLX; Prozac) on the autonomic control of resting HR, HRV (time and frequency domain), inotropic and chronotropic responses to sympathetic stimuli and chronotropic responses to vagal efferent stimulation, as well as indexes of sympathetic drive, including plasma catecholamines and LF blood pressure variability. Based on recent literature suggesting that depressed patients have lower HRV, in part, due to chronic treatment with SSRIs, we hypothesized that chronic SSRI treatment in CHF would further reduce HRV.

MATERIALS AND METHODS

Animals. All experiments were performed in accordance with the American Physiological Society's "Guiding Principles in the Care and Use of Animals" as well as "The Guiding Principles for Research Involving Animals and Human Beings" and were approved by the Institutional Animal Care and Use Committee of Loyola University. Male Sprague-Dawley rats between 300 and 350 g (Harlan, Indianapolis, IN) were acclimated to the vivarium for 1 wk before surgery while given ad libitum access to food and water. Rats were housed at a constant temperature of $22 \pm 2^\circ\text{C}$ with a 12:12-h light-dark cycle.

Coronary artery ligation. Animals were anesthetized with ketamine-xylazine (100 mg/kg + 7 mg/kg im), intubated, and ventilated with room air supplemented with 100% O_2 . Coronary artery ligation (CAL) surgery and sham surgery were performed as previously described (18). Rats were given lidocaine (10 mg/kg sc) just before ligation and every 2 h for 8 h after ligation to reduce the incidence of arrhythmia. All rats were given buprenorphine (50 $\mu\text{g/kg}$ sc) after arousal from surgery and again 18 h later.

Echocardiography. Rats were anesthetized with ketamine-xylazine (100 mg/kg + 7 mg/kg im) and subjected to echocardiography (Acuson Sequoia C256, Siemens) to determine left ventricular (LV) function 1 and 7 wk after sham operation or real CAL as previously described (18). All sham-operated rats and those CAL rats with fractional shortening (FS) between 12% and 25% were implanted with radiotelemetry probes for further study.

Telemetry probe implantation. In total, 18 sham-operated rats and 30 CAL rats were instrumented with radiotelemetry probes (C50-PXT, Data Sciences, St. Paul, MN) to enable 24-h sampling of blood pressure, HR, ECG, and locomotor activity as previously described (18). Probes were implanted subcutaneously under ketamine-xylazine anesthesia (100 mg/kg + 7 mg/kg im). Rats were given buprenorphine (25 $\mu\text{g/kg}$ sc) upon waking from anesthesia. Data acquisition began 1 wk after telemetry probe implantation.

Data acquisition and analysis. Telemetry recordings were acquired continuously over a 24-h period, once per week for 5 wk, using Dataquest A.R.T. 3.1 Gold software (Data Sciences). Variables were recorded at 1,000 Hz and analyzed as a moving average. ECG waveforms were analyzed with Chart version 5.2.2 and HRV Module version 1.1 (ADInstruments, Colorado Springs, CO).

HRV was determined from 5-min segments of ECG data. The choice of segments used for analysis was based on the relative lack of movement artifacts and arrhythmias. Arrhythmic beats (beats not initiated in the atria, as determined by visual inspection of the ECG) were manually removed. SD of normal-normal intervals (SDNN) was determined from 5-min segments of data from each hour and averaged over the course of 24 h to determine the average SD of normal-normal

intervals (SDANN). Fast Fourier transformation of the same 5-min segments of ECG data was performed after the removal of linear trends and application of a Welch window with a fast Fourier transform setting of $n = 1,024$ points with 50% overlap. Spectral power was quantified within the following frequency bands: LF power, 0.06–0.6 Hz; and HF power, 0.6 to 3.00 Hz.

Spontaneous baroreflex sensitivity (sBRS) was determined from 5-min, simultaneously recorded segments of the ECG R-R interval (RRI) and mean blood pressure (MBP) data obtained during the dark cycle between 9:00 and 11:00 PM since animals showed the lowest tendency toward ectopic beats and aberrant ECGs during this period. ECG data were resampled at 500 Hz followed by the manual removal of artifacts and analyzed with Nevrokard SA-BRS software (version 3.2.4) to determine sBRS by the sequence method. The average slope was obtained from linear regressions of the interbeat interval and mean systolic blood pressure acquired from a minimum of three sequences that satisfied the following constraints: three or more consecutive RRIs with a variation of >0.5 ms in the same direction that correlated with mean arterial pressure (MAP; $r^2 > 0.85$) variations of >0.5 mmHg, with a three-beat delay, as previously validated (18). Coherence between RRI and MBP variability was determined as the square root of the ratio of the RRI and MBP power spectra with a segment length of 128 points, 50% overlap, and zero padding of 8. The average coherence in the LF and HF domains was calculated as area under the curve within the specified domains. Systolic blood pressure variability was determined in the LF domain and in the time domain, the latter of which was determined as the SD of blood pressure between normal beats.

Drugs and autonomic blockade. Rats were given either fluoxetine [FLX; fluoxetine HCl (20 mg/kg sc), Sigma Aldrich] or vehicle (bacteriostatic H_2O) based on matched FS values determined 1 wk after ligation surgery. In a preliminary study (27), rats given $10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of FLX for 4 wk had FLX concentrations of 63.2 ± 17.7 ng/ml, lower than plasma concentrations observed in humans receiving 20–80 mg/day of FLX (100–700 ng/ml). Therefore, the dose was doubled to $20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, a dose similar to that found to be most effective in producing anxiolytic-like behavior in mice (11). Rats were given FLX or vehicle daily for 5 wk, starting the third week after CAL or sham CAL surgery. On the last day of the experiment, 2-h continuous baseline telemetric recordings were made, after which rats were injected with (\pm)propranolol HCl (4 mg/kg ip, Sigma Aldrich). After 2 h, when the HR response to propranolol was maximal, rats were injected with atropine methyl nitrate (2 mg/kg ip, Sigma Aldrich), and recordings were continued for 2 h. The following day, the reverse protocol was used, except that propranolol (4 mg/kg ip) was injected 15 min after atropine to ensure that atropine was still effective at the time of testing. HRV was determined in 5-min segments of ECG data taken before blockade, directly before the administration of the second drug and during the maximal response to the second drug, i.e., 1 h after propranolol or 20 min after atropine administration.

LV pressure recordings and cardiac receptor stimulation. LV pressure (LVP) and the contractility index $[(\text{dP}/\text{dR})/\text{LVP}$ at peak $\text{dP}/\text{dR}]$ were determined under ketamine-xylazine (100 mg/kg + 7 mg/kg im) anesthesia using a 2-Fr Millar pressure catheter (SPD-320, Millar Instruments) inserted through the right carotid artery. After the stabilization of LVP and HR, electrical stimulation was applied to the cervical vagus nerve as a square-wave pulse generated by a constant-current stimulator (Pulsar 6i, Frederick Haer & Company). A stimulus of 20 s in duration at an intensity of 500 μA was applied with a pulse duration of 1 ms at increasing frequencies (4, 8, 16, and 32 Hz) in 4-min intervals. Subsequently, rats were injected with increasing concentrations of isoproterenol (Isuprel, Hospira, 2.5×10^{-6} – 2.5×10^{-2} mg/kg iv).

Plasma catecholamine and FLX/norfluoxetine determinations. FLX and its major metabolite, norfluoxetine (NFLX), were determined by HPLC (53). Rats were euthanized with ketamine-xylazine

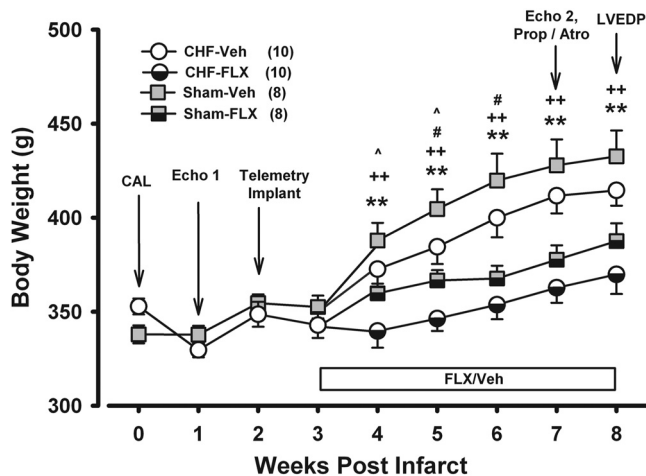


Fig. 1. Experimental timeline and body weight of congestive heart failure (CHF) and sham-operated (sham) rats that received vehicle (Veh) or fluoxetine (FLX; 20 mg·kg⁻¹·day⁻¹) for 5 wk (as indicated by the horizontal bar). CAL, coronary artery ligation; Echo, echocardiogram; Prop/Atro, propranolol/atropine blockade experiment; VS/ISO, vagal stimulation/isoproterenol injection experiment; LVEDP, left ventricular (LV) end-diastolic pressure. Values are group means \pm SE; numbers in parentheses are numbers of rats/group. ** P < 0.01 between CHF groups; ++ P < 0.01 between sham groups; ^ P < 0.05 between FLX-treated groups; # P < 0.05 between Veh-treated groups.

(100 mg/kg + 7 mg/kg im). Trunk blood was collected in EDTA, centrifuged, aliquoted, and frozen at -80°C until analysis. For analysis, 140 μl of internal standard [clomipramine (27.5 $\mu\text{g}/\mu\text{l}$), Sigma Aldrich], deionized H₂O (400 μl), and 1 M NaOH (1 ml) were added to 1 ml of plasma and vortexed. FLX and NFLX were extracted from the alkalized plasma with 2.5 ml *n*-hexane-isoamyl alcohol (99:1) followed by rocking for 5 min and centrifugation at 1,000 *g* for 10 min. The organic layer was removed, after which FLX and NFLX were back extracted by the addition of 100 μl of 0.05% phosphoric acid followed by rocking for 5 min and centrifugation at 1,000 *g* for 10 min. The aqueous layer was extracted for HPLC analysis (20 μl). The mobile phase consisted of MeCN-0.05 M KH₂PO₄ (pH 4.7; 40:60). Samples were run across a Supelcosil LC-18-DB column (15 cm \times 4.6 mm, 5 μm , Sigma Aldrich) in a Hewlett-Packard Series 1100 HPLC with an autoinjector and DAD detector (Agilent Technologies, Santa Clara, CA) at a flow rate of 2 ml/min. The column temperature was 50°C, and the detection wavelength was 214 nm. Samples were normalized to the internal standard (clomipramine) and compared with standard curves generated by spiking 1 ml of pooled plasma from sham-operated vehicle-treated rats with final concentrations of 0, 0.5, 1.0, and 2.0 $\mu\text{g}/\text{ml}$ NFLX and 0, 0.25, 0.5, and 1.0 $\mu\text{g}/\text{ml}$ FLX in 400 μl deionized H₂O. Run-to-run coefficients of variation were <10% with both 1 μg NFLX and 0.5 μg FLX.

Plasma norepinephrine and epinephrine concentrations were determined from aliquots of plasma collected in the same manner as described above in a subset of CHF rats treated with vehicle of FLX using methods previously described in detail (18).

Statistical analysis. Three-way ANOVAs with repeated measures were used to determine the effects of ligation and FLX on body weight, blood pressure, HR, locomotor activity, SDANN, power in the LF and HF domains, sBRS, cardiac contractility index, and HR responsiveness between groups. Two-way ANOVAs were used to assess the effects of ligation and FLX on terminal LV end-diastolic pressure (LVEDP), FS, lung weight-to-body weight ratio, heart weight-to-body weight ratio, SDNN, vagal and sympathetic control of HR, sBRS, and coherence, as well as the EC₅₀ and maximal elastance (E_{max}) of changes in the LV contractility index to vagal nerve stimulation and isoproterenol injection. Followup Bonferroni post hoc tests were used when appropriate for further analysis of group differences. P < 0.05 was considered significant.

RESULTS

Body weight. Analysis of body weight during exposure to FLX or vehicle in CHF and sham-operated rats (from week 3 through week 8) demonstrated a significant attenuation of weight gain among FLX- and vehicle-treated CHF groups that persisted throughout the experiment (P < 0.05 and P < 0.01, respectively; Fig. 1). FLX-treated rats showed both decreased overall body weight and an attenuated weight gain throughout the study (P < 0.01).

Echocardiography, HRV, and hemodynamic measurements. Only those rats that underwent CAL and showed elevated LVEDP and a lung-to-body weight ratio of >2 SD from the mean of sham-operated vehicle-treated rats (LVEDP > 8.5 mmHg, lung-to-body weight > 5.3 gm/kg) were included in the study. Of the 30 CAL rats, only 20 rats were determined to have developed CHF by the end of the study, based on these criteria. Of the 10 rats subjected to CAL that were not included in the analyses, one rat from each treatment group died before the end of the study and 4 rats from each group failed to develop CHF. Thus, FLX did not affect survival or CHF development. Consequently, rats with CHF had increased LVEDP 8 wk after ligation (P < 0.01) and decreased FS 7 wk after ligation (P < 0.01) compared with treatment-matched, sham-operated rats (Table 1). There was no effect of FLX on either parameter. Rats with CHF had increased lung- and heart weight-to-body weight ratios compared with their respective sham-operated groups given the same treatment (P < 0.01). FLX treatment increased the lung-to-body weight ratio in CHF rats (P < 0.01), but the tendency of this parameter to increase in sham-operated rats failed to reach significance. Plasma concentrations of FLX and its active metabolite, NFLX, were not affected by the surgical manipulation (Table 2). Plasma norepinephrine and epinephrine were only measured in a subset of CHF rats, so direct comparisons with sham-operated rats could not be made. There was no effect of FLX on plasma catecholamines (Table 3).

Table 1. Terminal parameters of congestion and ventricular function

	<i>n</i>	Left Ventricular End-Diastolic Pressure, mmHg	Fractional Shortening, %	Lung Weight-to-Body Weight Ratio, g/kg	Heart Weight-to-Body Weight Ratio, g/kg
Sham-operated vehicle-treated group	8	2.9 \pm 1.0	38.5 \pm 2.6	4.5 \pm 0.1	3.1 \pm 0.1
Sham-operated FLX-treated group	8	4.0 \pm 1.5	40.0 \pm 2.1	7.2 \pm 0.3	3.3 \pm 0.1
CHF vehicle-treated group	10	22.2 \pm 1.9*	11.6 \pm 0.9*	10.6 \pm 0.7*	4.8 \pm 0.1*
CHF FLX-treated group	10	23.6 \pm 1.7*	14.1 \pm 1.7*	14.9 \pm 1.1*†	5.1 \pm 0.2*

Values are group means \pm SE; *n*, no. of rats/group. FLX, fluoxetine; CHF, congestive heart failure. * P < 0.01 vs. the respective sham-operated group; † P < 0.01 vs. the CHF vehicle-treated group.

Table 2. Terminal plasma concentrations of FLX and NFLX

	<i>n</i>	NFLX, ng/ml	FLX, ng/ml
Sham-operated FLX-treated group	14	1,518 ± 176	278 ± 65
CHF FLX-treated group	12	1,440 ± 117	225 ± 31

Values are group means ± SE; *n*, no. of rats/group. NFLX, norfluoxetine.

There was no effect of the day-night cycle on blood pressure. Therefore, data were pooled across all 24 time points for further analysis. MAP was decreased in CHF rats throughout the experiment ($P < 0.01$; Fig. 2). FLX treatment tended to reduce MAP in both sham-operated and CHF groups early on after treatment, but the effect waned with time, resulting in a drug × time interaction ($P < 0.01$). However, there were no significant differences between FLX-treated rats and their respective vehicle-treated control groups at any time point throughout the experiment. FLX treatment tended to decrease HR in both sham-operated and CHF rats during the first 3 wk of treatment (Fig. 2). The effect was much more pronounced and only significant in sham-operated animals. HR returned to pretreatment levels in both groups by the end of the study. There was no difference in locomotor activity between groups (data not shown).

Rats with CHF had decreased SDANN 3 wk after CAL surgery ($P < 0.01$; Fig. 3A). After 5 wk of drug treatment (initiated after the week 3 measurement), there was a main effect of FLX ($P < 0.05$) due to a reduction of SDANN in both CHF and sham-operated groups, although only CHF rats demonstrated a significant difference from their vehicle-treated control group at week 8 ($P < 0.05$). The LF-to-HF ratio did not differ between groups 3 wk after surgery (before the start of FLX; Fig. 3B). FLX tended to reduce the LF-to-HF ratio in sham-operated rats and significantly reduced the ratio in CHF rats ($P < 0.05$) by the end of the study.

Autonomic blockade. The effects of autonomic blockade on MAP are shown in Table 4. Propranolol alone had no effect on blood pressure in any group. However, it consistently reduced pressure after atropine administration, although this effect was not significant in CHF rats treated with FLX. Atropine alone produced a substantial pressor effect in all groups except CHF rats treated with FLX. Atropine also increased blood pressure when given after propranolol in all groups except CHF rats treated with FLX.

Baseline HR did not differ between groups (Fig. 4A). Given that propranolol alone had no effect on blood pressure and so likely did not influence cardiac vagal tone by virtue of baroreceptor unloading, the vagal influence on HR was determined as the increase in HR produced by atropine administration in rats pretreated with propranolol. However, since atropine alone had a large effect on blood pressure that could have contributed to the baroreceptor-mediated withdrawal of sympathetic tone,

the sympathetic contribution to baseline HR was estimated by the fall in HR after propranolol alone. Using this scheme, it was noted that rats with CHF had reduced vagal control of HR ($P < 0.05$; Fig. 4B). FLX also reduced vagal control of HR in both sham-operated rats ($P < 0.01$) and CHF rats ($P < 0.05$). FLX increased the sympathetic contribution to HR, although the effect was only significant in CHF rats ($P < 0.01$). The relative contribution of vagally mediated and sympathetic activity on HR obtained by this method could be assessed by comparing the extent of the HR response above and below the zero point shown in Fig. 4B. The validity of determining the relative contribution of vagal and sympathetic tone to baseline HR using this method was verified by comparing baseline HR with intrinsic HR (Fig. 4C). As shown, the majority of the autonomic control of HR was vagal in sham-operated vehicle-treated rats. This assumption was verified by observation of a higher intrinsic HR in this group compared with their baseline HR. Likewise, FLX-treated groups had more sympathetic control of HR relative to their respective surgical vehicle-treated control groups. In accord, intrinsic HR was lower in FLX-treated groups.

Analyses of HRV in the frequency domain are shown in Fig. 5, A–D. The results when propranolol was given first are shown in Fig. 5, A and B, whereas data obtained when atropine was given first are shown in Fig. 5, C and D. Baseline LF HRV was reduced in CHF rats ($P < 0.01$) and with FLX treatment ($P < 0.01$), although the already low levels of LF HRV in CHF rats prevented any additional detectable effect of FLX. Propranolol

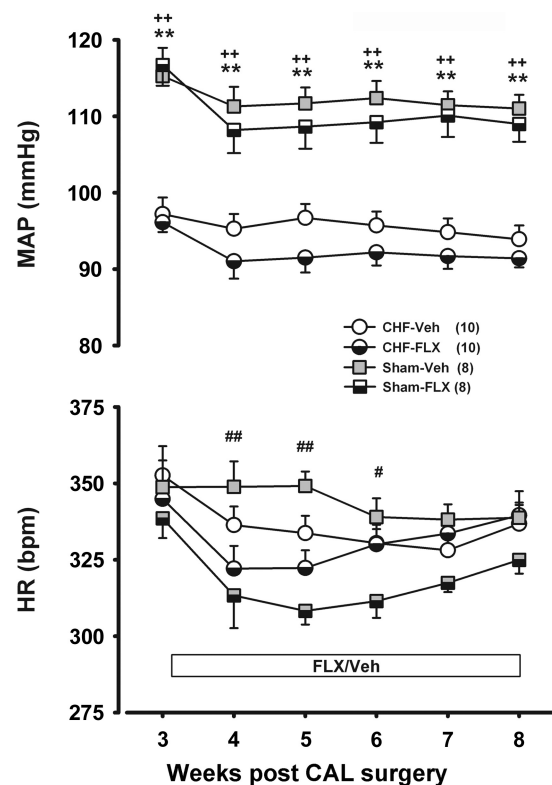


Fig. 2. Mean arterial pressure (MAP) and heart rate [HR; in beats/min (bpm)] in CHF and sham rats that received Veh or FLX for 5 wk. Values are group means ± SE; numbers in parentheses are numbers of rats/group. ** $P < 0.01$ between Veh-treated groups; + $P < 0.01$ between FLX-treated groups; # $P < 0.05$ and ## $P < 0.01$ between sham groups.

Table 3. Terminal plasma concentrations of norepinephrine and epinephrine in CHF rats that received vehicle or FLX treatment

	<i>n</i>	Norepinephrine, pg/ml	Epinephrine, pg/ml
CHF vehicle-treated group	7	2,086 ± 262	734 ± 67
CHF FLX-treated group	4	1,817 ± 273	958 ± 322

Values are group means ± SE; *n*, no. of rats/group.

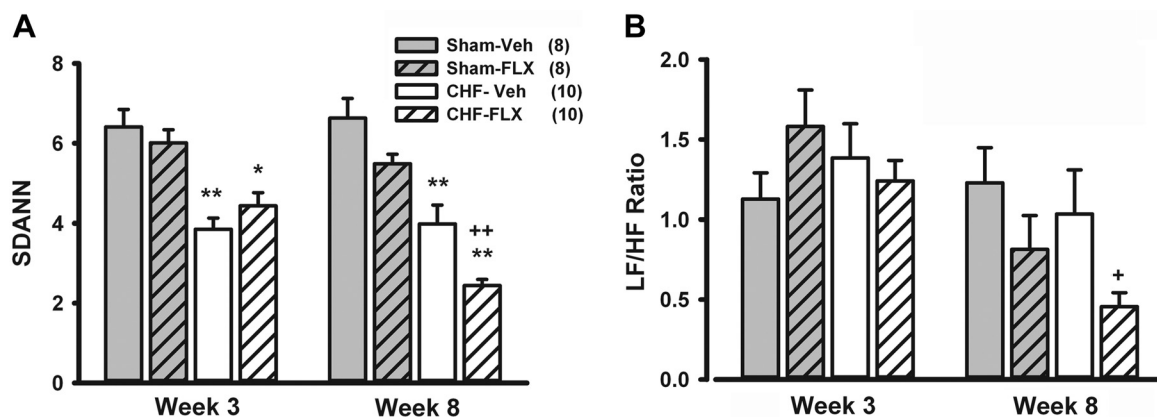


Fig. 3. Average SD of normal-normal intervals (SDANN; A) and LH-to-HF ratio (B) at 3 and 8 wk after sham or CAL surgery in rats that received 5 wk of Veh or FLX treatment. Drug treatment was initiated 1 day after the week 3 time point. Values are group means \pm SE; numbers in parentheses are numbers of rats/group. * $P < 0.05$ and ** $P < 0.01$ vs. the respective sham-operated group within the same week; + $P < 0.05$ and ++ $P < 0.01$ vs. the Veh-CHF group at 8 wk.

lowered LF power in both sham-operated groups ($P < 0.01$). Followup ANOVA demonstrated a larger effect of propranolol in sham-operated vehicle-treated rats compared with sham-operated FLX-treated rats ($P < 0.05$). Similarly, a significant interaction between FLX and propranolol was observed in CHF rats due to a larger drop in LF HRV after propranolol in vehicle-treated rats compared with that observed in FLX-treated rats. Group means did not differ after propranolol. Atropine had no further effect on LF HRV in any group.

A main effect of CHF on baseline HRV in the HF domain was evident ($P < 0.01$) due to the lower overall HF power in both CHF groups. However, only vehicle-treated rats showed a significant difference with CHF. Propranolol had no significant effect on HF HRV in any group. Atropine reduced HF power in sham-operated vehicle-treated rats when given after propranolol ($P < 0.01$), but it had no effect in any other group.

A similar pattern of group differences in baseline LF and HF HRV was observed on the day when atropine was given first. When given first, atropine significantly reduced LF HRV in sham-operated rats but had no effect on LF HRV in any other group. Propranolol further reduced LF power only in sham-operated rats treated with FLX ($P < 0.05$). Likewise, in the HF domain, atropine only significantly reduced HF HRV in sham-operated vehicle-treated rats ($P < 0.01$). Neither CHF groups nor the sham-operated FLX-treated group showed a significant effect of atropine in the HF domain. Propranolol had no additional effect on HF power after atropine.

sBRS, HR, and blood pressure variability. The number of sequences that met baroreflex criteria was reduced in rats with CHF 3 wk after ligation, before the start of FLX treatment (Fig. 6A). The number of detected sequences was reduced overall by 8

wk ($P < 0.01$). However, only sham-operated rats treated with FLX showed a significant within-group decline from 3 wk, and there was no difference between groups 8 wk after surgery. Although baroreflex gain of the detected sequences was reduced in CHF rats overall, between-group differences were only significant by 8 wk after surgery (Fig. 6B). FLX had no effect on spontaneous baroreflex gain.

Coherence between RRI and MAP was decreased in the LF domain in CHF rats 3 wk postsurgery, before FLX treatment ($P < 0.01$; Fig. 7A). Vehicle- and FLX-treated CHF rats also had decreased LF coherence compared with treatment-matched sham-operated groups at week 8 ($P < 0.01$; Fig. 7, B and C). There were no differences in coherence in HF HRV between any groups. However, there was a loss of the prominent 1- to 1.5-Hz respiratory-related peak in CHF rats at both 3 and 8 wk postsurgery.

Systolic blood pressure variability was reduced in CHF rats when assessed both in the time and LF domain 3 and 8 wk after ligation (Table 5). FLX treatment had no effect on blood pressure variability.

Cardiac sensitivity to autonomic stimulation. FLX treatment almost completely abolished the frequency-dependent negative chronotropic response to vagal efferent nerve stimulation in both CHF and sham-operated rats (Fig. 8 and Table 6). The significant interaction between surgery and drug treatment was due to a much larger suppression of responsiveness by FLX in sham-operated rats compared with CHF rats, particularly at the highest stimulus frequency. Vagal stimulation had no apparent effect on the ventricular contractility index in any group (data not shown).

Table 4. MAP after blockade of cardiac autonomic receptors

	n	MAP, mmHg					
		BL	Propranolol	Propranolol + atropine	BL	Atropine	Atropine + propranolol
Sham-operated vehicle-treated group	8	102 \pm 1	106 \pm 2	139 \pm 4§	105 \pm 2	132 \pm 3‡	119 \pm 5§
Sham-operated FLX-treated group	8	109 \pm 2	108 \pm 4	130 \pm 4§	110 \pm 4	125 \pm 5‡	111 \pm 3§
CHF vehicle-treated group	10	92 \pm 2	93 \pm 3*	103 \pm 3*§	92 \pm 2*	101 \pm 3*†	91 \pm 3*§
CHF FLX-treated group	10	90 \pm 1*	87 \pm 2*	94 \pm 2*	90 \pm 2*	94 \pm 3*	86 \pm 2*

Values are group means \pm SE; n, no. of rats/group. MAP, mean arterial pressure; BL, baseline. * $P < 0.01$ vs. the respective sham-operated group; † $P < 0.05$ and ‡ $P < 0.01$ vs. BL within group; § $P < 0.01$ vs. the first drug within group.

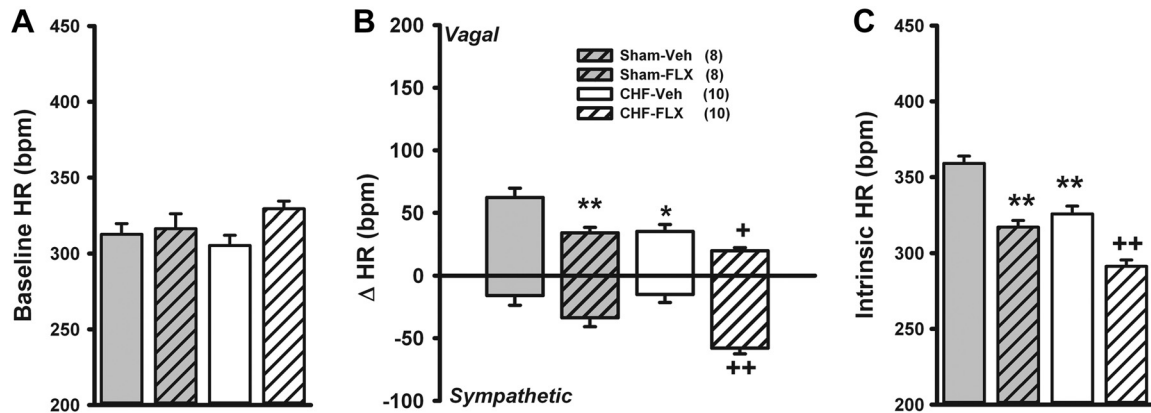


Fig. 4. Baseline HR (A), change in (Δ)HR in response to atropine treatment after sympathetic blockade (vagal) and to propranolol treatment alone (sympathetic) (B), and intrinsic HR (C) in CHF and sham rats after 5 wk of Veh or FLX treatment. Values are group means \pm SE; numbers in parentheses are numbers of rats/group. * P < 0.05 and ** P < 0.01 vs. the sham Veh-treated group; + P < 0.05 and ++ P < 0.01 vs. the CHF Veh-treated group.

Rats with heart failure showed a rightward shift in the chronotropic and inotropic response to isoproterenol (Fig. 9 and Table 6). FLX treatment tended to reduce HR E_{\max} . However, the difference was only significant in CHF rats. Isoproterenol produced a dose-dependant increase in the ventricular contractility index that was greatly attenuated in CHF rats (Fig. 9). The EC_{50} of the isoproterenol-induced increase in the ventricular contractility index was shifted to the right in CHF rats, but only FLX-treated groups differed significantly. E_{\max} was reduced in both CHF groups. FLX had no effect on the contractility index.

DISCUSSION

The present study demonstrated a profound effect of FLX on the autonomic control of HR both in sham-operated rats and rats subjected to chronic cardiac ischemia sufficient to cause CHF. Both FLX and CHF reduced the sympathetic contribution to LF HRV. The reduction in LF HRV in CHF was most likely mediated by loss of baroreflex modulation of sympathetic cardiac tone. The mechanism by which FLX lowered LF HRV remains less clear, although the data suggest that FLX might have reduced β -adrenergic receptor-mediated responses,

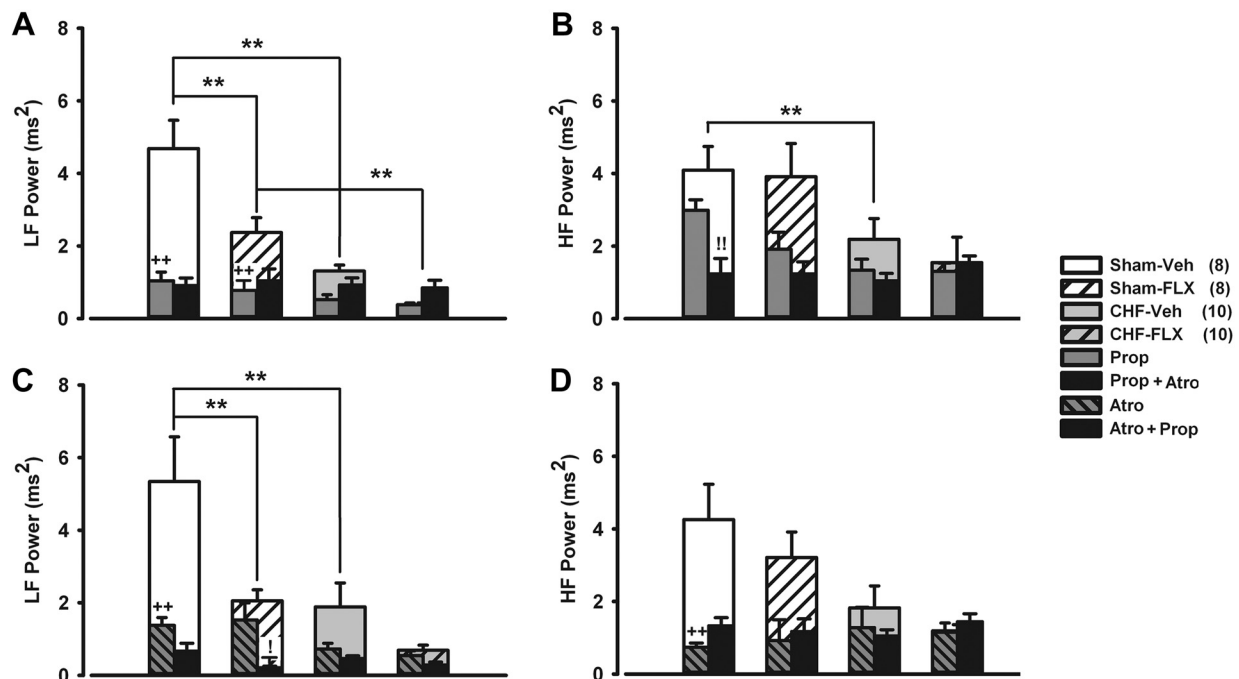


Fig. 5. A and B: low-frequency (LF; A) and high-frequency (HF; B) HR variability (HRV) before propranolol (background bars) and after propranolol (left bar inside background bar) and subsequent dual blockade with propranolol and atropine (right bar inside background bar) in CHF and sham rats after treatment with Veh or FLX for 5 wk. C and D: LF (C) and HF (D) HRV before atropine (background bars) and after atropine (left bar inside background bar) and subsequent dual blockade with atropine and propranolol (right bar inside background bar) in CHF and sham rats after treatment with Veh or FLX for 5 wk. Values are group means \pm SE; numbers in parentheses are numbers of rats/group. ** P < 0.01 between baseline values before drug administration; ++ P < 0.01, within group between baseline and the first drug treatment; ! P < 0.05 and !! P < 0.01, within group between the first and second drug treatment.

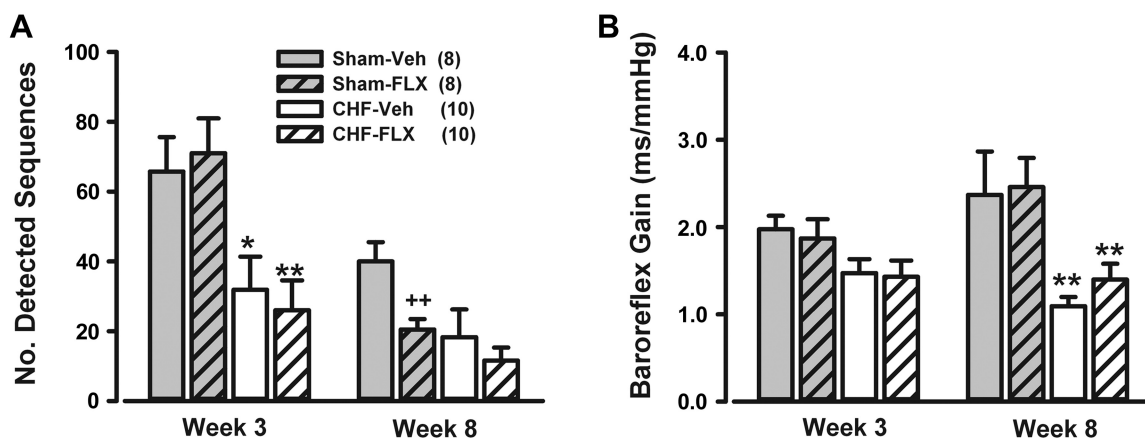


Fig. 6. *A*: number of sequences detected met the baroreflex criterion in CHF and sham rats that received Veh or FLX 3 and 8 wk postsurgery. *B*: baroreflex gain of sequences detected in CHF and sham rats that received Veh or FLX 3 and 8 wk postsurgery. Values are group means \pm SE; numbers in parentheses are numbers of rats/group. * $P < 0.05$ and ** $P < 0.01$ vs. the respective sham group; ++ $P < 0.01$ vs. week 3 within group.

particularly in rats with CHF. Both FLX and CHF also reduced HF HRV by decreasing muscarinic receptor-mediated responses. Both CHF and FLX reduced intrinsic HR. However, normal baseline HR was maintained by reduced vagal and/or increased sympathetic tone in both CHF and FLX-treated rats. Although FLX and CHF had profound effects on the parasympathetic and sympathetic control of HR, only CHF rats showed impaired autonomic control of ventricular function. Together, these findings indicate that chronic FLX may further impair the already compromised autonomic control of sinus rhythm in CHF.

Decreased HRV is associated with increased morbidity and mortality in CHF (26, 47). Up to 40% of CHF patients experience depression and anxiety, both of which are also associated with reduced HRV and an increased risk of death due to cardiovascular events (10, 20–22, 37, 55). Reduced HRV may signal an increased susceptibility to arrhythmogenesis and sudden cardiac death (15, 25). Whether pharmacological treatment of mood disorders improves HRV or cardiovascular outcomes in patients with cardiovascular disease remains controversial (14, 23, 29). Interpretation of these studies is difficult due to the use of differing depression scales, inclusion of patients with widely varying levels of ventricular dysfunction, different methods of HRV analysis, and the concomitant use of antidepressants with other drugs that influence autonomic function, including β -blockers and other antiarrhythmic drugs. This is the first assessment of the effects of SSRIs on autonomic cardiac control in a heart failure model without such confounds.

In the present study, as in our previous study (18), rats with CHF showed a significant loss of HRV when assessed either in the time or frequency domain. Contrary to our findings, a pilot study (29) based on a relatively small sample size showed that 12 mo of treatment with the SSRI sertraline increased SDNN in heart failure patients without comorbid depression. However, sertraline did reduce LF HRV in this patient population. In our study, propranolol produced a substantially smaller decrease in LF HRV after FLX treatment, suggesting that the decrease in LF HRV observed with chronic SSRI treatment may be mediated by loss of sympathetic modulation of LF oscillations. However, observations of a similar decrement of LF HRV after atropine, coupled with a complete lack of effect

of atropine in sham-operated FLX-treated rats, suggests that decreased parasympathetic modulation of LF HRV also contributes to the ability of FLX to diminish LF HRV. In our study, baroreflex-mediated withdrawal of sympathetic activity during the pressor response to atropine likely contributed to atropine's effects on LF HRV in control animals. FLX-treated animals showed a smaller pressor effect of atropine than vehicle-treated control animals (15 vs. 27 mmHg) and so likely experienced less baroreflex-mediated sympathetic withdrawal with atropine administration. In accord, propranolol had no effect on LF HRV in sham control rats when given after atropine, whereas FLX-treated rats showed a significant loss of LF HRV with the addition of propranolol. Thus, sympathetic withdrawal due to atropine was likely diminished in FLX-treated rats as a result of the smaller pressor effect of atropine in this group. These data provide compelling evidence that chronic SSRI treatment reduced the sympathetic modulation of LF HRV. At the same time, FLX greatly attenuated the bradycardic responses to efferent vagal stimulation, suggesting that loss of muscarinic receptor sensitivity or cell surface expression may partly account for the diminished effect of atropine on HRV in sham-operated FLX-treated rats.

It remains to be determined whether FLX reduced LF HRV by reducing sympathetic input to the heart or by altering cardiac autonomic receptor sensitivity or expression. FLX had no effect on circulating catecholamines, which suggests that the treatment did lower sympathetic drive in general. However, plasma catecholamine levels represent a composite of adrenergic neurotransmission, including neurotransmitter release, degradation, and reuptake in several vascular beds, and so may not reflect sympathetic output to the heart. Indeed, our study suggests that sympathetic tone to the heart was actually elevated with FLX, as indicated by the trend for a larger fall in HR after propranolol in sham-operated FLX-treated rats that was exaggerated in rats with CHF. It is not clear why sympathetic-dependent LF HR oscillations were reduced with FLX, whereas sympathetic control of HR appeared to be increased. This phenomenon has been described in human CHF and has been attributed to a loss of baroreflex modulation of HR after reductions in β -adrenergic receptor expression in the heart due to sustained increases in sympathetic cardiac drive (44a). However, there was no apparent effect of FLX on sBRS. Our

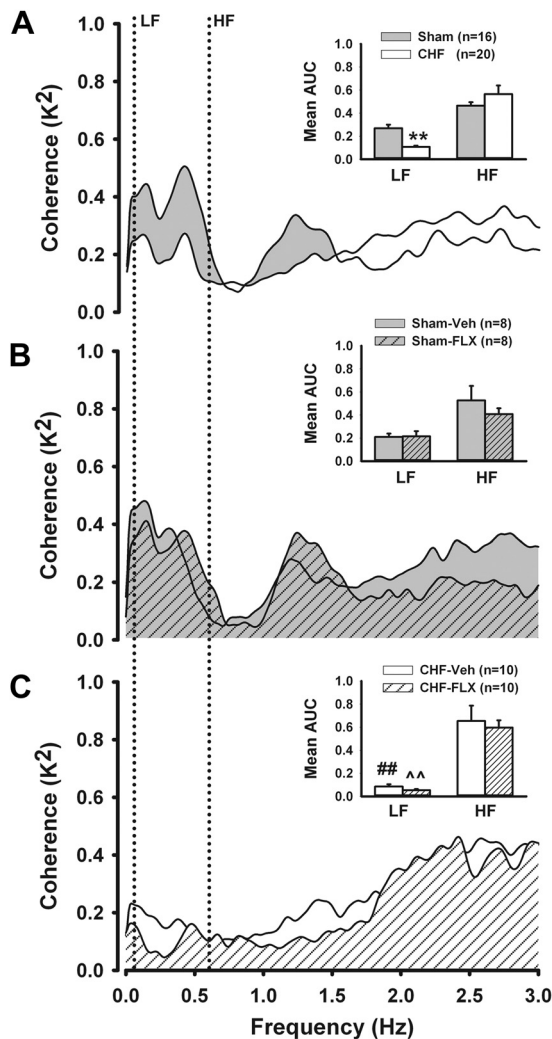


Fig. 7. *A*: coherence between R-R interval and MAP variability over a range of frequencies in CHF and sham rats 3 wk after surgery and before drug treatment. *B* and *C*: coherence in sham rats (*B*) and CHF rats (*C*) after 5 wk of Veh or FLX treatment. Lines are group means. Insets show coherence averaged across the LF and HF domains. AUC, area under the curve. Inset values are group means \pm SE. ** $P < 0.01$ vs. sham rats; ## $P < 0.01$ vs. the 3-wk CHF Veh-treated group; ^^ $P < 0.01$ vs. the 3-wk CHF FLX-treated group.

evidence showing that FLX reduced the maximal HR response to isoproterenol in sham-operated rats suggests that FLX treatment may have reduced sinoatrial node β -receptor expression and/or coupling. However, the decrement in efficacy was only found at very high doses of isoproterenol when HR was

increased by >120 beats/min. A recent study (6) also showed that 21 days of FLX attenuated the tachycardic response to hypotension in conscious unrestrained rats. Again, the most prominent effect of FLX was only observed after relatively large pressure changes. The ability of FLX to attenuate the chronotropic effects of isoproterenol were much more pronounced in rats with CHF. Among these animals, the effects could be seen at doses that caused HR changes of only 40 beats/min. As such, the extent of the effect of FLX on β -receptor function appears to be insufficient to contribute to the loss of LF oscillations at baseline blood pressure in sham-operated rats but may have contributed to the complete abolition of any LF oscillations in CHF rats. Indeed, FLX had no effect on LF coherence between blood pressure and interbeat interval among sham-operated animals but did produce a decrease in coherence among CHF rats.

Given the significant pressor effects of atropine, we chose to estimate the sympathetic contribution to HRV from data obtained when propranolol was given before atropine. Propranolol had no significant effect on blood pressure in any group, and, as such, its effects were likely not confounded by baroreflex alterations in parasympathetic activity. As expected, propranolol when given alone clearly decreased LF HRV in control animals. It also produced a limited, nonsignificant reduction in HF HRV. Although atropine continued to raise blood pressure after propranolol administration, use of the associated change in HRV to assess the vagal component of HRV was considered valid since blockade of all autonomic input to the heart would preclude the effects of baroreflex activation from confounding changes in HRV measures. As expected, atropine produced a large fall in HF HRV in control animals, confirming the well-known contribution of parasympathetic activity to respiratory sinus arrhythmia (7). Atropine had virtually no effect on HF HRV when given after propranolol in FLX-treated rats. When atropine was given first, FLX-treated rats also failed to show a significant decline in HF HRV from baseline. These findings further substantiate evidence showing that chronic FLX treatment produced an antimuscarinic effect. Desensitization of central muscarinic receptors has been suggested as a mechanism of action for the antidepressive and anxiolytic effects of SSRIs (12). Interestingly, elevated HF HRV in female bulimic patients is normalized after chronic FLX treatment (41). More recent studies (9, 17, 30, 32) have demonstrated that chronic SSRI treatment may contribute to the findings of lower HRV in patients with depression and anxiety. It is not clear why FLX lowers muscarinic receptor-mediated responsiveness since the drug shows a low affinity

Table 5. BP variability parameters between vehicle- and FLX-treated sham-operated and CHF rats at weeks 3 and 8 after coronary artery ligation

	<i>n</i>	Week 3		Week 8	
		BP SDNN, ms	LF power, mmHg ²	BP SDNN, ms	LF power, mmHg ²
Sham-operated vehicle-treated group	8	3.10 \pm 0.19	6.98 \pm 1.03	3.33 \pm 0.25	8.23 \pm 2.13
Sham-operated FLX-treated group	8	3.54 \pm 0.34	8.35 \pm 1.34	3.47 \pm 0.30	9.33 \pm 2.35
CHF vehicle-treated group	10	2.06 \pm 0.07†	3.00 \pm 0.39	2.08 \pm 0.11*	3.08 \pm 0.33†
CHF FLX-treated group	10	2.20 \pm 0.17*	4.05 \pm 1.35*	2.03 \pm 0.13*	2.59 \pm 0.50*

Values are group means \pm SE; *n*, no. of rats/group. Blood pressure (BP) variability was determined from systolic blood pressure waveforms by the spectral method. BP SDNN, SD of the beat-to-beat intervals of pressure; LF, low frequency (0.06–0.6 Hz). * $P < 0.01$ and † $P < 0.05$ vs. the respective sham-operated group.

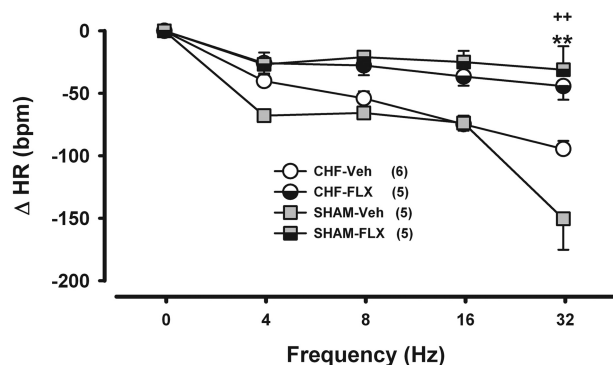


Fig. 8. Δ HR with increased frequency of efferent vagal nerve stimulation. Values are group means \pm SE; numbers in parentheses are numbers of rats/group. $**P < 0.01$ between CHF groups; $++P < 0.01$ between sham groups.

for muscarinic receptors (44). To our knowledge, no studies have yet assessed the effects of chronic FLX on muscarinic receptor function in the brain, nor is it known whether chronic FLX alters muscarinic receptor density or function in atrial tissue.

As in our prior study (18), CHF significantly attenuated HF HRV. The present study confirmed that this effect was due to loss of parasympathetic-mediated HF oscillations given that HRV in the HF domain did not decrease further in CHF animals given atropine after propranolol. FLX also attenuated the vagal modulation of HF HRV in sham-operated rats, as evidenced by the lack of any change in HF HRV after atropine administration in rats given prior propranolol. The very limited amount of HF HRV present in CHF rats precluded FLX from having further observable effects in this group. The attenuated bradycardic effect of vagal efferent stimulation found in FLX-treated rats suggests that insensitivity to neurotransmitter is an important mechanism behind the loss of parasympathetic-dependent HF HRV.

In contrast to human heart failure, rats with CAL-induced heart failure typically do not exhibit tachycardia (18, 46). This appears to be due, in part, to the reduced intrinsic HR that masks sympathetic-mediated tachycardia. Intrinsic HR is reduced in other models of heart failure as well. Sinoatrial node pacemaker cells isolated from volume- and pressure-overloaded rabbits show decreases in the hyperpolarization-activated pacemaker current and in the slow component of the delayed rectifier current, both of which contribute to slowed diastolic depolarization (50). Our findings indicate that reductions in intrinsic HR in animals with CHF were compensated by a deficit in tonic vagal drive to the heart. FLX treatment further exaggerated the loss of intrinsic HR in CHF rats.

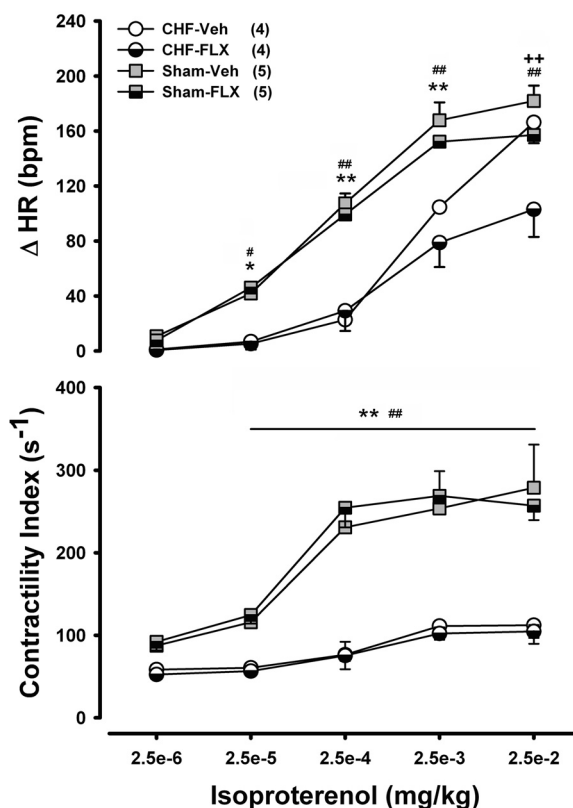


Fig. 9. Δ HR (A) and ventricular contractility index (B) with increasing doses of isoproterenol. Values are group means \pm SE; numbers in parentheses are numbers of rats/group. $*P < 0.05$ and $**P < 0.01$ between Veh-treated groups; $\#P < 0.05$ and $##P < 0.01$ between FLX-treated groups; $++P < 0.01$ between sham groups.

However, with FLX, HR was normalized by both decreased vagal tone and increased sympathetic tone. The effects of FLX on intrinsic pacemaker currents in the heart are virtually unknown.

The concentration of plasma FLX observed in treated rats from the present study was within the range reported in humans receiving 20–80 mg/day of FLX (27). However, the plasma NFLX concentration was much higher in our rats compared with that reported for humans. This is due to the more efficient hepatic metabolism of FLX to NFLX in the rat as well as the longer half-life of NFLX. Both FLX and NFLX are effective serotonin reuptake inhibitors. However, the *R*-enantiomer of NFLX is almost 20 times less potent than the *S*-enantiomers of FLX and NFLX or the *R*-enantiomer of FLX, all of which are equipotent to each other (51, 52). Thus, the higher metabolic breakdown of FLX renders the combined effect of FLX and

Table 6. Dose-response parameters for the chronotropic and inotropic actions of isoproterenol

	<i>n</i>	Change in Heart Rate		Contractility Index	
		EC ₅₀ , μ g/kg	<i>E</i> _{max} , beats/min	EC ₅₀ , μ g/kg	<i>E</i> _{max} , s ⁻¹
Sham-operated vehicle-treated group	5	0.26 \pm 0.06	198 \pm 11	0.07 \pm 0.01	283 \pm 33
Sham-operated FLX-treated group	5	0.12 \pm 0.02	164 \pm 6	0.05 \pm 0.01	264 \pm 5
CHF vehicle-treated group	4	2.13 \pm 0.14 [†]	184 \pm 8	0.43 \pm 0.10	114 \pm 11 [†]
CHF FLX-treated group	4	1.13 \pm 0.50*	108 \pm 22* [‡]	0.44 \pm 0.16*	106 \pm 4*

Values are group means \pm SE; *n*, no. of rats/group. Contractility index, [(dP/dt)/left ventricular pressure at peak + dP/dt]; *E*_{max}, maximal elastance. $*P < 0.05$ vs. the sham-operated FLX-treated group; $^{\dagger}P < 0.01$ vs. the sham-operated vehicle-treated group; $^{\ddagger}P < 0.01$ vs. the CHF vehicle-treated group.

NFLX somewhat less potent in rats than in humans given that a much higher portion of combined active agents is in a lower potency form in the rat. When the plasma concentrations of FLX and NFLX were summed and the expected concentration of the racemic mixture adjusted for the relative potencies of the constituents, it was found that the predicted serotonin reuptake inhibitory effects of FLX treatment in rats fell within the upper range of that predicted for humans taking a normal 20- to 80-mg daily dose of FLX (27). In the rat, subcutaneous injection of FLX reaches its peak plasma concentration within 1 h, whereas NFLX reaches its peak after 6 h (40). The half-life of an acute dose of FLX is reportedly 5.9 h in the rat (5). With chronic administration, however, the half-life of FLX is increased (16). Given that the plasma concentrations of FLX and NFLX were determined ~6 h after subcutaneous injection in our study, the plasma concentrations we observed likely reflected the upper portion of the normal fluctuation range for FLX and peak concentrations of NFLX. Thus, the effects we observed with our dosing regimen should be comparable with those observed in individuals taking clinically relevant doses of FLX.

In summary, our study demonstrated that when given alone without other drug regimens, chronic FLX produces a complex set of autonomic responses that culminate in reduced HRV in both healthy rats and those with CHF. The overall loss of HRV after chronic FLX administration can be attributed to the loss of both vagal and sympathetic mediated oscillations. Loss of vagally mediated oscillations is related to reduced muscarinic receptor sensitivity. In CHF rats, the loss of LF oscillations with FLX appears to be related to a loss of sensitivity of β -receptors. However, the mechanism responsible for the loss of sympathetic-mediated variability in sham-operated rats treated with FLX awaits future studies that can address the receptor signaling mechanisms responsible for these observations.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.H., R.L.T., and K.E.S. conception and design of research; M.H., R.L.T., A.M.S., and E.H. performed experiments; M.H., A.M.S., E.H., and K.E.S. analyzed data; M.H., R.L.T., A.M.S., E.H., and K.E.S. interpreted results of experiments; M.H. and K.E.S. prepared figures; M.H. and K.E.S. drafted manuscript; M.H., A.M.S., E.H., and K.E.S. edited and revised manuscript; M.H., R.L.T., A.M.S., E.H., and K.E.S. approved final version of manuscript.

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