Impaired serotonergic regulation of heart rate may underlie reduced baroreflex sensitivity in an animal model of depression

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Hildreth CM, Padley JR, Pilowsky PM, Goodchild AK. Impaired serotonergic regulation of heart rate may underlie reduced baroreflex sensitivity in an animal model of depression. Am J Physiol Heart Circ Physiol 294: H474–H480, 2008. First published November 9, 2007; doi:10.1152/ajpheart.01009.2007.—Serotonin (5-HT) is crucial to normal reflex vagal modulation of heart rate (HR). Reduced baroreflex sensitivity [spontaneous baroreflex sensitivity (sBRS)] and HR variability (HRV) reflect impaired neural, particularly vagal, control of HR and are independently associated with depression. In conscious, telemetered Flinders-Sensitive Line (FSL) rats, a well-validated animal model of depression, we tested the hypothesis that cardiovascular regulatory abnormalities are present and associated with deficient serotonergic control of reflex cardiovascular function. In FSL rats and control Flinders-Resistant (FRL) and Sprague-Dawley (SD) rat strains, diurnal measurements of HR, arterial pressure (AP), activity, sBRS, and HRV were made. All strains had normal and similar diurnal variations in HR, AP, and activity. In FRL rats, HR was elevated, contributing to the reduced HRV and sBRS in this strain. In FSL rats, sBRS and high-frequency power HRV were reduced during the night, indicating reduced reflex cardiovascular activity. The ratio of low- to high-frequency bands of HRV was increased in FSL rats, suggesting a relative predominance of cardiac sympathetic and/or reflex activity compared with FRL and SD rats. These data show that conscious FSL rats have cardiovascular regulatory abnormalities similar to depressed humans. Acute changes in HR, AP, temperature, and sBRS in response to 8-hydroxy-2-(di-n-propylamino)tetralin, a 5-HT1A, 5-HT1B, and 5-HT7 receptor agonist, were also determined. In FSL rats, despite inducing an exaggerated hypothermic effect, 8-hydroxy-2-(di-n-propylamino)tetralin did not decrease HR and AP or improve sBRS, suggesting impaired serotonergic control of cardiovascular activity. These data suggest that impaired serotonergic control of cardiac reflex function could be one mechanism linking reduced sBRS to increased cardiac risk in depression.

serotonin-1A receptors; 8-hydroxy-2-(di-n-propylamino)tetralin; Flinders Sensitive Line rat; vagus; telemetry

Tonic and reflex control of heart rate (HR) is dependent on the balance of parasympathetic (vagal) and sympathetic neural input. Disruption of this balance, through a decrease in vagal tone or an increase in sympathetic tone, leads to cardiac dysfunction and/or arrhythmias (35, 43).

Tonic levels of cardiovagal activity and, consequently, the level of resting HR are controlled by the activity of cardiac vagal preganglionic neurons (CVPN) located in the nucleus ambiguus (NA) and dorsal motoneuron of the vagus (44). Within the NA, the rhythmic activity of CVPN is dependent on excitatory and inhibitory synaptic inputs (22, 44), including ongoing baroreflex mediated via the nucleus of the solitary tract (NTS) and respiratory input (9, 33). Phasic inhibition of inhibitory input to CVPN is critical for producing reflex-mediated bradycardia (46). Intravenous injection of the serotonin (5-HT)-1A (5-HT1A) partial agonist buspirone potentiates the reflex increase in vagally mediated bradycardia that occurs in response to baroreceptor loading or stimulation of cardiopulmonary receptors in anesthetized animals (37). Intracisternal administration of WAY-100635, a selective 5-HT1A antagonist, attenuates the reflex bradycardia (13, 37). Thus it has been suggested that 5-HT in the NA may provide phasic inhibition of inhibitory inputs to CVPN (12).

Reduced spontaneous baroreflex sensitivity (sBRS) and HR variability (HRV) are associated with impaired cardiac autonomic balance in hyper tension, coronary artery disease, and myocardial infarction (2a, 14, 15). Recently, the impact of affective disorders, such as depression, on cardiovascular outcome has been examined. Depressive symptoms are associated independently with reduced sBRS (3) and HRV (1, 2) and increased risk of sudden cardiac death in patients with coronary artery disease (8) and after myocardial infarction (17). The biological mechanisms leading to impaired autonomic control of the heart in depression are unknown. Disruption of 5-HT neurotransmission plays a major role in the etiology of depression: 5-HT levels (20) and 5-HT receptor abnormalities similar to depressed humans. Acute changes in HR, AP, temperature, and sBRS in response to 8-hydroxy-2-(di-n-propylamino)tetralin, a 5-HT1A, 5-HT1B, and 5-HT7 receptor agonist, were also determined. In FSL rats, despite inducing an exaggerated hypothermic effect, 8-hydroxy-2-(di-n-propylamino)tetralin did not decrease HR and AP or improve sBRS, suggesting impaired serotonergic control of cardiovascular activity. These data suggest that impaired serotonergic control of cardiac reflex function could be one mechanism linking reduced sBRS to increased cardiac risk in depression.

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The Flinders-Sensitive Line (FSL) rat, bred for cholinergic hypersensitivity, is a well-validated genetic animal model of depression exhibiting a number of behavioral and neurochemical (11, 47, 48) similarities to depressed humans. Recently, we demonstrated reduced sBRS and HRV in anesthetized FSL rats compared with control strains (28), closely mimicking cardiovascular regulatory abnormalities in depressed humans. Here we hypothesized that conscious FSL rats would exhibit abnormalities of cardiovascular control associated with abnormal 5-HT1A receptor control of cardio- vagal outflow.

To test this hypothesis, we made 24-h recordings of arterial pressure (AP) and HR in conscious FSL rats and its inbred, Flinders-Resistant Line (FRL) rat, and outbred control, Sprague-Dawley (SD) rat, strains. We examined diurnal patterns of AP, HR, and activity, sBRS, HRV, and parasympathetic and sympathetic contributions to HR were examined during the plateau phases of the day-light cycle. We then examined the autonomic effects evoked by acute administration of the 5-HT1A, 5-HT1B, and 5-HT7 agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT).
METHODS AND MATERIALS

Animals

All experiments were approved by the Royal North Shore/University of Technology Sydney Animal Care and Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Male FSL rats (n = 12, 10–14 wk old) and age-matched controls, FRL (n = 11) and SD (n = 10) rats, were used. At 1 wk before experimentation, the centrally evoked hypothermic response to oxotremorine (0.2 mg/kg ip) was tested. As reported previously (28), FSL rats exhibited much larger hypothermic responses (2.5 ± 0.1 vs. 0.5 ± 0.1°C in FSL vs. FRL, P < 0.0001).

Telemetry System

A Datalab (IV) telemetry system (Data Sciences) with two transmitters (models TA11PA-C40 and C50-PXT) was used. Both models permitted measures of AP and activity; C50-PXT transmitters also measured temperature and ECG.

Surgical Procedures

Rats were housed individually and placed on weaner food and water (amoxicillin trihydrate 9.3 mg/l and tylosin tartrate 6.2 mg/l) 1 wk. Animals were anesthetized with pentobarbitone sodium (60 mg/kg ip) and carprofen (2.5 mg ip), a nonsteroidal analgesic, and cephalosporin, a cephalosporin antibiotic, was administered. After anesthetization was assessed regularly using reflex responses to tactile (corneal stroking) and noxious (hindpaw pinch) stimuli. Additional doses of pentobarbitone sodium (6 mg ip) were administered as required. Aseptic surgical techniques were used throughout the procedure. Telemetry transmitters were implanted into the scapula region under halothane anesthesia (1.2% halothane, 0.01% atropine) were administered using osmotic minipumps (Alzet) implanted subcutaneously in the scapular region under halothane anesthesia. Diurnal Changes in HR, AP, and activity. Parameter data were exported offline in 1-h bins to examine diurnal variation. HR data were also averaged between 11 AM–1 PM and 11 PM–1 AM. These time periods were chosen, inasmuch as they reflect plateau phases of the HR circadian rhythms and, therefore, minimized variations in HR due to physical activity, feeding, and the sleep cycle (42). GraphPad Prism (version 4.0) was used for statistical analysis.

HRV. Spectral analysis of short-term AP waveform recordings was used to examine HRV. In the rat, periodic oscillations of HR occur at low and high frequencies (LF and HF, respectively) (28, 32). HF oscillations are synchronous with respiratory frequency and represent vagally mediated modulation of HR, also termed respiratory sinus arrhythmia (2a, 38). LF oscillations are closely associated with the baroreflex and may be due to combined vagal and sympathetic influences on HR (2a, 38).

Waveform data were imported offline into Spike CED (version 6.0). Pulse interval (PI) time series were derived offline from the AP waveform. PI was uniformly resampled at 10 Hz, and HRV indexes were calculated from the PI time series between 11 AM–1 PM and 11 PM–1 AM during periods when animals were quiescent. Criteria for choosing segments for HRV analysis were stationarity and lack of ectopic beats. Four 80-s epochs were chosen for analysis, and the average value of these was used for each animal. Power spectra were derived using fast Fourier transformation (size 256, Hann window), giving a final frequency resolution of 0.04 Hz. LF and HF powers were calculated within the frequency ranges 0.25–0.75 Hz and 1–3 Hz, respectively, and the LF-to-HF ratio (LF/HF) was calculated from these data.

HR baroreflex sensitivity. Waveform data were imported offline into Spike 2. sBRS was calculated, using the sequence method (28), as the slope of the relationship between a rise in mean AP (MAP) and lengthening of the PI (+MAP/+PI) or a decrease in MAP and shortening of the PI (−MAP/−PI). Criteria of three consecutive beats with a +MAP/+PI or −MAP/−PI relationship and a change in MAP of ≥1.5 mmHg were used. Where continuous AP recordings were made, sBRS was calculated over a 5-min period; 80-s epochs were used when data were acquired noncontinuously over a 24-h period. The average slope of all lengthening and shortening sequences between 11 AM–1 PM and 11 PM–1 AM was used as an estimate of sBRS in the day and night, respectively. The average slope of all lengthening and shortening sequences over three 5-min segments before and after 30 min after 8-OH-DPAT injection was calculated to assess the effects of 8-OH-DPAT on sBRS.

Statistical analysis. Values are means ± SE. One-way ANOVA with Bonferroni’s correction was used for group comparisons. Two-way ANOVA was used to assess the effects of time on HR, MAP, and activity over the 24-h period. P < 0.05 was considered significant. The relationship between HF power and resting HR was analyzed by fitting linear (y = mx + b) or nonlinear [y = span.exp(−kx)] regression lines.

RESULTS

Diurnal Changes in HR, AP, and Activity

All rats exhibited nocturnal elevations in HR, MAP, and activity (P < 0.001; Fig. 1A). Over the 24-h period, resting HR was higher in FSL (P < 0.05; Fig. 1A) and SD (P < 0.001; Fig. 1A) rats. HR did not differ between FSL and SD rats over the 24-h period (P > 0.05; Fig. 1A). MAP was elevated in FRL compared with FSL rats (P < 0.05; Fig. 1A). MAP did not differ between SD and FSL or FRL rats (Fig. 1A).

Activity did not differ between the strains over the 24-h period (Fig. 1A).
Contribution of Parasympathetic and Sympathetic Tone to Resting HR

A 2-h window during day and night plateaus was used to assess parasympathetic and sympathetic contributions to HR. The differences between resting HR under methylatropine or metoprolol and during complete autonomic blockade were determined. During the day and night, methylatropine had similar effects in all strains (Fig. 2A). During the day, metoprolol had a much larger effect in FSL than FRL (P < 0.05) and SD (P < 0.05) rats, indicating a greater sympathetic contribution to HR in this strain (Fig. 2B). Under complete autonomic blockade, resting HR was higher in FRL rats during the day (333 ± 7 vs. 289 ± 7 beats/min in FRL vs. SD, P < 0.01) and night (385 ± 6 vs. 336 ± 9 beats/min in FRL vs. SD, P < 0.01) than in SD rats and during the night than in FSL rats (385 ± 6 vs. 342 ± 10 beats/min in FRL vs. FSL, P < 0.05).

sBRS

sBRS was significantly reduced in FSL and FRL compared with SD rats (P < 0.05) at night (Fig. 3A). No differences in sBRS were found in any strain during the day (Fig. 3A).

HRV

Similar patterns of LF, HF, and LF/HF spectral components were seen during the day and night in each strain. LF power did not differ between strains (Fig. 3B). HF power was reduced in FSL (P < 0.05) and FRL (P < 0.01) compared with SD rats during the night (Fig. 3E). LF/HF was significantly higher in FSL than SD rats during the day and night (P < 0.05; Fig. 3C).

Cardiac sympathetic blockade with metoprolol did not affect HRV in the LF and HF ranges in any strain. HF power remained significantly reduced in FSL (1.2 ± 0.2 vs. 5.2 ± 1.6 ms² in FSL vs. SD, P < 0.05) and FRL (0.9 ± 0.2 vs. 5.2 ± 1.6 ms² in FRL vs. SD, P < 0.01) rats compared with SD rats at night. Metoprolol significantly reduced LF/HF in FSL rats during the night (P = 0.0321; Fig. 3D). No significant differences in LF/HF existed between the FSL and SD rats after metoprolol (Fig. 3D).

The relationship between HF power and HR was determined in all strains, and three distinct relationships were revealed when data from all time periods were pooled (Fig. 3F). HR ranged from 260 to 470 beats/min in SD and FSL rats and from 320 to 510 beats/min in FRL rats. Nonlinear and linear regression showed an inverse correlation between HR and HF power that was significantly nonzero in SD and FRL rats (P = 0.016), respectively. An explanation for the linear relationship in FRL rats may be that the range of HR available did not include any between 250 and 320 beats/min. In contrast, HF power in FSL rats did not exhibit a relationship with HR; HF power was similar throughout the range of HR investigated (P = 0.936).

Fig. 2. Over a 2-h period during the day (11 AM–1 PM) and night (11 PM–1 AM) average HR changes due to methylatropine (A) or metoprolol (B) were calculated with respect to complete autonomic blockade in FSL, FRL, and SD rats. Metoprolol evoked a much greater effect in FSL rats during the day. *P < 0.05.
Effect of Acute Injection of 8-OH-DPAT on MAP, HR, Temperature, and sBRS

In SD rats, 8-OH-DPAT produced a transient increase in MAP and HR, attributable to the stress of intraperitoneal injection (36), followed by a marked decrease in MAP, HR, and temperature (Fig. 4A). Similar time courses and magnitudes of the 8-OH-DPAT-induced reductions in MAP, HR, and temperature were seen in FRL and SD rats (P < 0.05; Fig. 4C). A similar stress-induced transient tachycardia and pressor response were also seen in the FSL rats (Fig. 4B and C). In contrast, FSL rats exhibited a marked hypothermia but no reduction in MAP or HR (Fig. 4B and C) after the stress response. 8-OH-DPAT-induced hypothermia was greater in FSL than FRL (P < 0.001) and SD (P < 0.001) rats.

The effects of 8-OH-DPAT on sBRS were analyzed in all rats 30 min after injection (Fig. 4D). 8-OH-DPAT significantly increased sBRS in FRL (P = 0.002) and SD (P = 0.017) rats (Fig. 4D). In contrast, 8-OH-DPAT did not induce a change in sBRS in FSL rats (P = 0.21; Fig. 4D).

**DISCUSSION**

The three major findings of this study are as follows. 1) Conscious FSL rats exhibit cardiovascular reflex abnormalities (reduced HRV and sBRS during the night), but basal levels of blood pressure and HR were similar to those in control strain(s). 2) 8-OH-DPAT decreases HR, AP, and temperature and enhances baroreflex control of HR (sBRS) in conscious SD and FRL rats. 3) 8-OH-DPAT evokes no tonic or reflex cardiovascular responses in FSL rats, despite an exaggerated hypothermic response. These findings suggest that abnormal serotonergic control of vagal input to the heart may contribute to increased cardiovascular risk.

We show that FSL, FRL, and SD rats have similar diurnal patterns of HR, AP, and activity. The higher resting HR in FRL rats than in the other two strains appears to be due to an elevated intrinsic (nonneural) HR. In FRL and FSL rats, sBRS and HF power are low during the night. Spontaneous indexes of HRV are influenced by sinus cycle length, such that spectral components of HRV, as well as BRS, are inversely related to resting HR (31, 49). We confirm that there is a normal inverse relationship between HF power and resting HR, such that as HR increases, HF power decreases, in FRL and SD rats. Inasmuch as the resting HR of the FRL rat is high, this accounts for the reduced HF power and sBRS in this strain.

Inasmuch as resting HR was similar in FSL and SD rats, the low sBRS, low HF power (during the night), and an atypical relationship between HF power and HR in FSL rats are indicative of abnormal reflex vagal function. We showed that metoprolol did not affect LF or HF power in any strain, confirming that both of these frequency ranges are predominantly under reflex vagal influence (32, 41). LF/HF is increased in FSL rats, suggesting, although perhaps contentiously, a
predominance of sympathetic activity at the sinoatrial node (18). The enhanced LF/HF in the FSL rat was reduced by metoprolol, indicating that β-adrenergic receptors play a role in its genesis. In support of this, the sympathetic/vagal contribution to resting HR was altered in the FSL rat at least during the day, indicating a relative predominance of sympathetic tone in this strain. Together, this suggests that the FSL rat has abnormal reflex vagal function and increased cardiac sympathetic activity.

The FSL rat is a well-validated animal model of depression, showing similar neurochemical (11, 47, 48) and behavioral correlates (25–27). Here we show conclusively that conscious FSL rats have a normal HR and blood pressure but reduced HF power, increased LF/HF, and reduced sBRS, particularly during their active period, and that these dysfunctions are similar to those in depressed patients during the day in populations with or without cardiovascular disease (1–3).

We investigated the effects of systemic 8-OH-DPAT, a selective 5-HT_{1A} receptor agonist, with some affinity for 5-HT_{1B} and 5-HT_{7} receptors, on the tonic and reflex control of autonomic function in the three rat strains. In control SD and FRL rats, 8-OH-DPAT evoked reductions in HR, AP, and temperature. The precise site of action of 8-OH-DPAT in this study cannot be ascertained but has previously been localized to activation of 5-HT_{1A} receptors within the central nervous system (4, 7, 45), presumably heteroreceptors within the rostral ventrolateral medulla (23) and NA (45). The reduction of temperature is thought to occur via activation of 5-HT_{1A} autoreceptors (21), potentially in the midline raphe (24, 39).

In control rats, we showed for the first time that 8-OH-DPAT also significantly enhanced sBRS (a measure of reflex vagal activity). This is in agreement with the findings of Skinner et al. (37), who demonstrated in atenolol-treated anesthetized rabbits that stimulation of 5-HT_{1A} receptors with buspirone enhanced baroreflex-mediated bradycardia. The NTS and NA are two central sites of action that could potentially mediate this increase in reflex vagal function. Intracerebrally applied selective 5-HT_{1A} or 5-HT_{7} antagonists reduce baroreflex sensitivity (13). 5-HT_{7} receptors are located only in the NTS (10); 5-HT_{1A} receptors are found in the NTS and NA (40). Iontophoretic application of the selective 5-HT_{1A} antagonist WAY-100635 onto CVPN in the NA attenuates their activation as a result of stimulation of cardiopulmonary afferents. These data suggest that 5-HT_{1A} receptors in the NA, possibly acting presynaptically at GABA terminals (12), may mediate the enhanced sBRS seen here.
Low HRV and sBRS are directly linked to increased risk of cardiac mortality in depression (1–3). The mechanisms underlying abnormal regulation of HR in depression are unknown. The present study shows for the first time that the FSL rat, a genetic animal model of depression, exhibits reductions in sBRS and HRV and an increase in LF/HF closely resembling cardiovascular regulatory abnormalities evident in human depression. Furthermore, these reflex cardiovascular abnormalities in FSL rats were associated with impaired 5-HT₁A receptor control of cardiac vagal activity together with sympathetic function. Hence, impaired central 5-HT receptor control of cardiovagal activity together with sympathetic function as a possible explanation for the increased cardiac risk in depression. A significant decrease in 5-HT₁A mRNA (19) and a decrease in 5-HT₁A receptor levels (20) have been reported in hippocampus and cortex (heteroreceptors) (34) and significant increases have been reported in raphe regions (autoreceptors) (30) of nontreated depressed patients, although such discrimination has not been found in all studies (5). It is therefore possible that, in some depressed patients, abnormal 5-HT₁A receptor function is present in areas important for cardiovascular control. Potentially, this may occur at GABA synapses in the nucleus accumbens, leading to reduced phasic inhibition of CVPN and poor reflex control of HR.

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REFERENCES


