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Ondansetron modulates pharmacodynamic effects of ketamine on electrocardiographic signals in rhesus monkeys

Paolo B. DePetrillo [a,*], Allyson J. Bennett [b], d'Armond Speers [c], Stephen J. Suomi [d], Susan E. Shoaf [e], Kamran Karimullah [a], J. Dee Higley [b]

[a] *Laboratory of Clinical Studies, Unit of Clinical and Biochemical Pharmacology, Division of Intramural Clinical and Biochemical Research, National Institutes of Health, National Institute on Alcohol Abuse and Alcoholism, NIH 10 / 3C103, 10 Center Drive MSC 1256, Bethesda, MD20892-1256, USA*

[b] *Laboratory of Clinical Studies, the Primate Unit, Division of Intramural Clinical and Biochemical Research, National Institutes of Health, National Institute on Alcohol Abuse and Alcoholism, Poolesville, MD, USA*

[c] *Intelligent Systems, Thomson Labs, Rockville, MD 20850, USA*

[d] *Laboratory of Comparative Ethology, National Institutes of Health, National Institute on Child Health and Human Development, Poolesville, MD, USA*

[e] *Laboratory of Clinical Studies, Unit of Pharmacokinetic Studies, Division of Intramural Clinical and Biochemical Research, National Institutes of Health, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA*

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Abstract

Electrocardiographic signal dynamics were examined in rhesus monkeys (*Macaca mulatta*) before and after treatment with ketamine and/or ondansetron. Ketamine exerts differential pharmacodynamic effects on behavior in animals stratified according to a measure of central serotonergic turnover. We hypothesized that measures of serotonergic turnover might explain some of the variance in the electrocardiographic (ECG) response to ketamine. Electrocardiographic recordings of animals were obtained at baseline, after administration of either saline or ondansetron (0.125 mg/kg), and after administration of ketamine (15 mg/kg). Electrocardiographic signal dynamics were measured using an algorithm that extracts the Hurst parameter (H) of the interbeat interval (IBI) time-series. H decreased after ketamine administration, (mean ± S.E.M.), 0.33 ± 0.04 vs. 0.12 ± 0.02, $P \leq 0.001$, $n = 10$. Cerebrospinal fluid 5-hydroxyindole-3-acetic acid (5-HIAA) concentrations, a measure of serotonergic turnover, predicted the monkeys' response to ketamine, $H = 0.001$ (5-HIAA, pmol/ml)-0.130, $R = 0.66$, $P \leq 0.003$, $n = 18$. Ondansetron attenuated the response to ketamine, 0.14 ± 0.02 vs. 0.08 ± 0.02, $P \leq 0.05$, $n = 8$, ondansetron vs. saline. These data provide evidence that naturally occurring differences in serotonin function alter the ECG response of the animals to ketamine and that activation of the serotonin type-3 receptor by ketamine is involved.

Keywords: Ketamine; Ondansetron; 5-HT (5-hydroxytryptamine, serotonin); Heart rate; Electrocardiography; Fractal

1. Introduction

The pharmacodynamic effects of ketamine and ondansetron alone and in combination on electrocardiographic (ECG) signal dynamics were investigated in rhesus monkeys. It was hypothesized that ketamine administration would strongly perturb cardiac signal dynamics because racemic ketamine antagonizes the *N*-methyl-D-aspartate (NMDA) receptor, blocks the serotonin transporter, and increases serotonin type-3 (5-HT_3) receptor-mediated Ca^{2+}-currents by a mechanism not dependent on inhibition of the serotonin transporter (Martin et al., 1988; Peters et al., 1991; Nishimura et al., 1998). Areas of the brain stem such as the nucleus tractus solitarius involved in heart rate regulation are rich in 5-HT_3 as well as excitatory amino acid receptors (Nieuwenhuys, 1985). Profound effects on the reflex regulation of heart rate are seen following pharmacologic manipulation of NMDA and 5-HT_3 receptor activities (Chianca and Machado, 1996; Sevoz et al., 1996, 1997; Lo et al., 1997; Pires et al., 1998).

Inter-individual differences in cerebrospinal fluid concentrations of 5-hydroxyindole-3-acetic acid (5-HIAA) explain some of the variance in the pharmacodynamic re-

* Corresponding author. Tel.: +1-301-496-9420; fax: +1-301-402-0445.

E-mail address: pbdp@helix.nih.gov (P.B. DePetrillo).

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sponse to ketamine. Ketamine-induced sleep time in rhesus monkeys is inversely proportional to cerebrospinal fluid 5-HIAA concentration, which is a measure of serotonin turnover in the central nervous system (Shannon et al., 1997). This measure is a stable trait in primates, allowing its use as a surrogate marker of serotonergic functioning (Higley and Linnoila, 1997, 1998). We hypothesized that ketamine would differentially alter cardiac signal dynamics in animals varying in degree of serotonergic turnover, since serotonergic activation is involved in regulation of heart rate. We therefore included measurements of cerebrospinal fluid 5-HIAA concentrations as a possible explanatory variable for variations in cardiac signal dynamics.

We measured cardiac signal dynamics by calculating the Hurst parameter (H) of the electrocardiographically derived interbeat interval (IBI) time-series. We used H as the pharmacodynamic outcome variable because it was found to be a more sensitive measure of drug-related cardiac effects in human subjects than measurements of heart rate magnitude alone (DePetrillo et al., 1999b). The Hurst parameter was conceived by Hurst (1951) and formalized by Mandelbrot and Van Ness (1968) as a way of measuring time-series dynamics. The value of H varies as $0 \leq H \leq 1$. As can be seen from the synthetic time-series in Fig. 1, when H approaches 0, rapid alterations in the magnitudes of the IBIs give the time-series a very rough texture as seen in the upper left of the figure. By contrast, as H approaches 0.5, there is less variation in the R–R IBIs and the time-series curves have a smoother appearance. Since all these time-series were designed to have the same mean and standard deviation, it is apparent that dispersional measures in the time domain are not sensitive indicators of underlying signal dynamics. We theorized that the pharmacodynamic effects of ketamine could be characterized at a higher resolution by H than by mean and standard deviation measures derived from groups of IBI data.

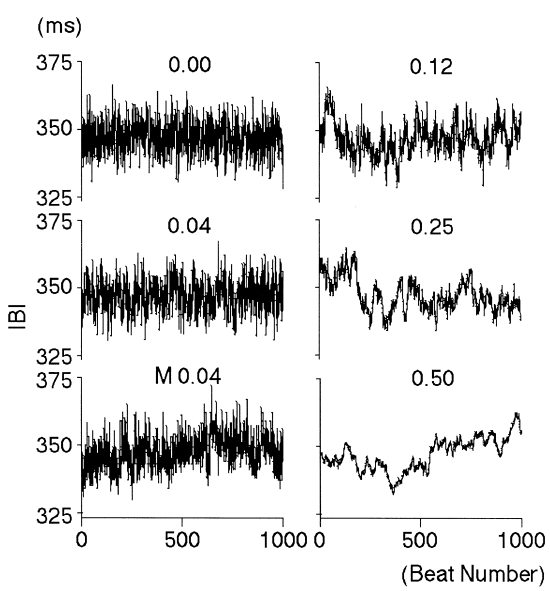

Fig. 1. IBI time series and associated H parameters. The synthetic time series were generated to have the same mean (346 ms) and S.D. (6) as the experimentally derived time-series M 0.04.

2. Materials and methods

2.1. Animal procedures

All procedures were approved by the NIAAA Animal Care and Use Committee (Protocol #LCS75 and LCS-AB-01). The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Animals were 10 male rhesus monkeys (*Macaca mulatta*) between 655 and 898 days of age ($M = 851$ days, S.D. = 74) at the onset of testing for the first experiment, and eight female rhesus monkeys ($M = 1128$ days, S.D. = 76) for the second experiment.

Animals were selected to represent the full range of CNS serotonin functioning on the basis of CSF 5-HIAA concentrations obtained prior to testing. These values ranged from 286 to 486 pmol/ml ($M = 365$, S.D. = 73) for the first experiment, and 155 to 292 pmol/ml ($M = 216$, SD = 47) for the second experiment, within the norms for 2-year-old rhesus monkeys (Higley et al., 1991).

Prior to the first test day, a 2-ml cisternal cerebrospinal fluid sample was collected from each monkey less than 20 min after it was anesthetized with ketamine hydrochloride (15 mg/kg i.m.). Previous studies (Bacopoulos et al., 1979; Brammer et al., 1987; Higley et al., 1996) have shown monoamine concentrations to be unaffected if cerebrospinal fluid samples are obtained within 25–30 min of capture. Samples were obtained with a 5-ml syringe and a 22-gauge needle, immediately aliquoted and frozen in liquid nitrogen, and then transferred to storage at -70°C. Cerebrospinal fluid samples were assayed for concentrations of serotonin metabolite 5-HIAA using high performance liquid chromatography with electrochemical detection and internal standardization (Scheinin et al., 1983). The within-day and between-day variations for the assay were 3% and 5%, respectively. The average within-animal correlation of serially sampled cerebrospinal fluid 5-HIAA over a space of 7 months showed $R = 0.80$.

2.2. Experimental procedure

ECG IBI data was collected as follows. To begin testing, an individual animal was hand-captured, removed from its homecage and physically restrained. The anterior chest wall was shaved beginning approximately at the mid-axillary line and proceeding to an area just below the

nipples on the left and right. Gel ECG electrodes (ConMed, Utica, NY, USA) were attached to the anterior chest wall, with the positive electrode placed at approximately 1-cm below and lateral to the left nipple, and the negative electrode placed just below the right nipple. Proper placement of the electrodes was documented using a CD-200 oscilloscope monitor. For some animals, better tracings were obtained with one or both leads placed on the limbs. Sometimes the electrode placement required moving the positive electrode medially to produce an adequate QRS voltage complex with positive deflection of the *R* wave. When the electrodes were securely placed, the animal was restrained on a plastic board with restraint straps. Electrodes were wired to a MM Polar XR transmitter in communication with a Mini-Logger Series 2000 receiver (Mini-Mitter, SunRiver, OR, USA), which was used to store the IBI data. The logger configuration file was adjusted in consultation with the manufacturer to allow reliable capture of IBIs corresponding to heart rates of up to 320 beats per min. The sampling rate for the device is 500 Hz and results in a timing accuracy of ≤ 1 ms in the measurement of the IBIs (Ruha et al., 1997).

The experimental design is illustrated in Fig. 2. In both experiments, the first 20 min of the heart beat IBI recording took place while the animal was awake but restrained on the board. Heavy cloth was placed over the animal's eyes to minimize distress and external stimuli. In the first experiment, 20 min of baseline recording was followed by a 15 mg/kg dose of ketamine hydrochloride administered via intramuscular injection into the upper posterior left thigh followed by 20 min of recording under anesthesia. In the second experiment, the animals were given either ondansetron 0.125 mg/kg or an equal volume of saline IV, and awake recording was continued for an additional 20 min, after which a 15 mg/kg dose of ketamine hydrochloride was administered. Monkeys were then recorded for the remaining 20 min while they were unconscious.

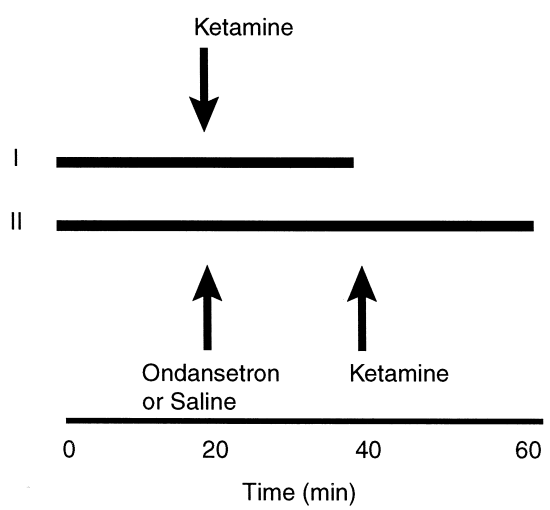

Fig. 2. Illustration of the experimental design. In experiment I, $n = 10$ animals were given ketamine (15 mg/kg) at the time indicated, while in Experiment II, $n = 8$ animals were given either ondansetron (0.125 mg/kg) or saline, followed by ketamine (15 mg/kg) at the time indicated.

Where necessary several minutes of recording time were added to the awake segment in order to compensate for data lost to bouts of movement. The dose of ketamine used resulted in complete loss of muscle tone in the animals for approximately 20 min and produced the level of anesthesia typically used for routine veterinary surgical procedures.

2.3. Data analysis

Cardiac IBI data, in milliseconds, were retrieved from the Mini-Logger receiver using Version 3.5 of the Mini-Logger software on an IBM 433DX/Dp Personal Computer running MS DOS 6.22. The IBI data were filtered using linear interpolation if any single IBI was more than twice the magnitude of the previous IBI. The maximum number of data points requiring adjustment for any IBI time-series examined were always less than 3.5% of the total number of IBI data points, and in most individual cases was less than 0.2%.

Data was analyzed as previously reported (DePetrillo et al., 1999a) using software incorporating an algorithm, which extracts the fractal dimension D of the time-series and derives the Hurst value as $H = 2 - D$. The software application used for these analyses, running on Windows (TM) 95, 98 or NT, can be obtained through a procedure outlined at ftp://helix.nih.gov/ pbdp/.

The value of the dimensional embedding constant used for the analysis was estimated by increasing the embedding dimension in increments of 1 until D reached a stable value. Empirical testing showed that a maximal embedding dimension of six resulted in a stable measure of D for all time-series tested.

An automatic procedure was used to slice the IBI time-series into 1000 data point segments. The second 1000 point segment was used to calculate the baseline H value. The first 1000 point segment that occurred at least 5 min after ondansetron/placebo and/or ketamine administration was used to calculate the post-ondansetron/placebo or post-ketamine H value. The time point obtained after the dose of ketamine point corresponded to the minimum value of H reached after ketamine administration.

A repeated-measures analysis-of-variance was calculated to determine whether there were significant differences in the values of H or IBI magnitude before and after ketamine administration. The resulting parameters were used to derive the P-values of the mean differences. Multiple linear regression models using baseline cerebrospinal fluid 5-HIAA concentration (pmol/ml), age at testing (days), and gender as the independent variables and measured value of H before and after ketamine administration as the dependent variables were calculated. Inde-

pendent variables were retained in the models with a value for retention set at $P \leq 0.05$.

3. Results

The R^2-value for the linear regressions used for the calculation of H values were all 1.00, suggesting that a strong scale-independent power law relationship (Peitgen et al., 1992) applies to the rhesus monkey IBI time-series. The relationship of the IBI time-series and the calculated H parameter is shown for one animal in Fig. 3.

A large decrease in H occurs after administration of ketamine, as shown in Figs. 4 and 5. In both sets of experiments, there were small but significant increases in IBI after ketamine administration when compared to baseline. Ondansetron attenuated the effects of ketamine on cardiac signal dynamics as measured by the H parameter. Compared to placebo, ondansetron pre-treatment increased post-ketamine H from 0.08 ± 0.02 to 0.14 ± 0.02 (M $\pm$ S.E.M.) as shown in Fig. 5.

Baseline cerebrospinal fluid 5-HIAA concentrations were correlated with post-ketamine H values, as shown in Fig. 6 but not with pre-ketamine H values, nor the pre-or post-IBI values, $P > 0.3$. A multiple linear regression calculated using the post-ketamine H value as the dependent variable and the baseline cerebrospinal fluid 5-HIAA concentrations and age at testing and gender as the independent variables was calculated. The results show that only baseline cerebrospinal fluid 5-HIAA concentration is a statistically significant predictor of H. Neither age nor gender were retained in the final model. The complete model is: measured value of H after ketamine administration $= 0.001$(5-HIAA, pmol/ml) $- 0.130$. ($R = 0.66$, $P \leq 0.003$). A parallel model using the same explanatory vari-

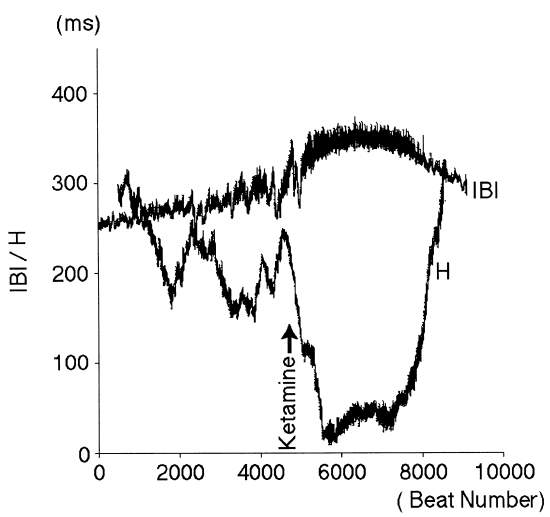

Fig. 3. The IBI time-series and corresponding H-values from one experimental animal are shown. The y-axis shows the magnitude of each IBI and value of $H \times 1000$. A window comprising 1000 data points is moved along the IBI time-series, the H-value is determined, and the window is advanced by one beat number. The process is repeated until the end of the IBI time-series. The H-values are anchored to the midpoint of the window of the IBI time-series. The arrow points to the beat number at which ketamine was given.

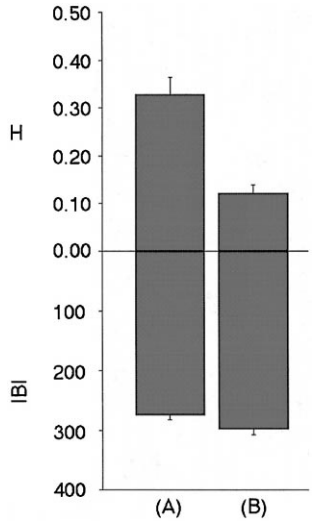

Fig. 4. Mean values of the Hurst parameter (H) and values of the IBI in millisecond are shown at baseline (A) and after ketamine administration (B) for $n = 10$ monkeys obtained from experiment I. The lines above and below the columns represent the positive displacement of the S.E.M. There are significant differences in the values of H and IBI before and after ketamine, (0.33 ± 0.04 vs. 0.12 ± 0.02, $p < 0.001$) and (271 ± 9 vs. 296 ± 11, $p < 0.03$) between the two conditions.

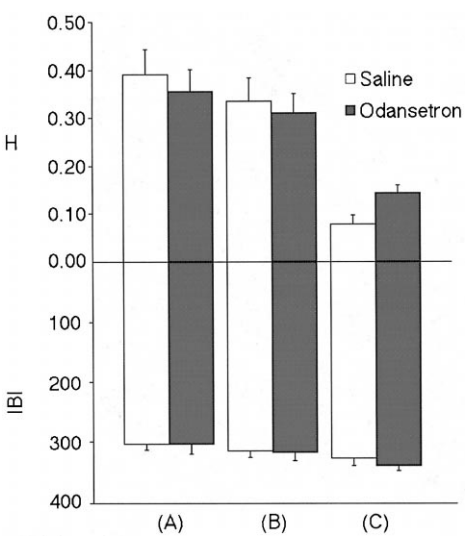

Fig. 5. Mean values of the Hurst parameter (H) and the values of the IBI in millisecond at baseline (A), after ondansetron or saline (B) and after ketamine (C) in $n = 8$ animals, obtained from experiment II. The light and dark columns represent the mean data obtained in the Saline or Ondansetron conditions, respectively. The lines above and below the columns represent the positive displacement of the S.E.M. There are no significant differences between Saline and Ondansetron conditions either IBI or H at (A) and (B). At the time point indicated by (C), $H = 0.08 \pm 0.02$ vs. $H = 0.14 \pm 0.02$, significantly different at $P \leq 0.04$ for Saline vs. Ondansetron.

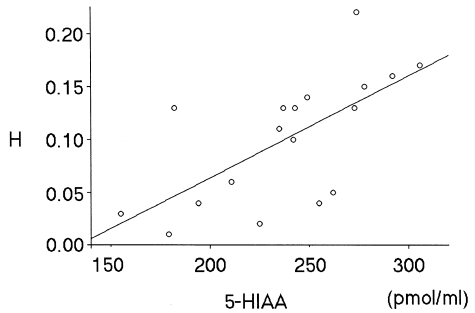

Fig. 6. Correlation between baseline cerebrospinal fluid 5-HIAA concentration and the value of the Hurst parameter (H) obtained after administration of ketamine. The linear regression value of H after ketamine administration = 0.001(5-HIAA, pmol/ ml) − 0.130. ($R = 0.66$, $P \leq 0.003$).

ables with H determined before ketamine administration was rejected.

4. Discussion

Ketamine administration decreased the value of H in all animals tested. Ondansetron, a 5-HT_3 receptor antagonist, attenuated these effects. Taken together, these results suggest that ketamine exerts some of its effects on cardiac signal dynamics via activation of 5-HT_3 receptors. These results parallel similar studies in mice, where ketamine was also found to increase R–R interval variability (Mitchell et al., 1998).

Our results demonstrate that interindividual differences in serotonergic activity are associated with differential responses to ketamine-induced alterations in cardiac signal dynamics. Following ketamine dosing, much of the variance in H could be explained by the previously obtained central nervous system serotonin turnover measurement. Lower indices of cerebrospinal fluid 5-HIAA concentrations were associated with increased values of H after ketamine. The variance of IBIs increases in humans after treatment with serotonin reuptake inhibitors such as paroxetine, fluoxetine and doxepin (Tucker et al., 1997; Khaykin et al., 1998). Taken together, these data point to an important role for serotonergic activation in the modulation of heart rate variability.

Higher 5-HT_3 receptor sensitivity induced by lower serotonergic exposure may have led to the increased cardiac response associated with ketamine in animals with naturally occurring low levels of synaptic serotonin, as reflected by low cerebrospinal fluid 5-HIAA concentrations. The sensitivity of 5-HT_3 receptors to agonist-induced channel-opening is known to be tightly regulated, and is dynamically altered by exposure to serotonin or other agonists via a reversible post-translational mechanism (Van Hooft and Vijverberg, 1995, 1997).

A faster metabolic rate of ketamine in the monkeys with high cerebrospinal fluid 5-HIAA concentrations might have accounted for differences in the cardiac response to ketamine. This is unlikely since the time points chosen for determination of H were obtained shortly after drug administration. Changes in levels of active drug would have been minimized even if a difference in drug metabolism was operating.

We included age as a possible explanatory variable because of the previous observations that cerebrospinal fluid 5-HIAA concentrations decrease with age (Higley et al., 1991). However, the age range in our sample may have been too small and the variance in 5-HIAA may have been too large for age to remain a significant explanatory variable in our model. Gender was also included as a possible explanatory variable because of reports that human heart rate variability may be increased in females as compared to males (Ryan et al., 1994). However, we failed to find a significant gender-related effect in 5-HIAA values, suggesting that hormonal environment may modulate central neural systems at a level which is not reflected in measures of serotonin turnover.

Serotonin differences were unrelated to both heart rate and the change in heart rate following ketamine administration ($P > 0.40$), suggesting that other factors may control heart rate and that these factors are dissociated from variability measures. Baseline cerebrospinal fluid 5-HIAA concentrations were also not correlated with H prior to the administration of ketamine. Since under these conditions the monkeys were awake, restrained, and under stress, it is possible that any serotonin-mediated difference in cardiac signal dynamics may have been attenuated by high levels of circulating catecholamines.

The results of this study support the use of the Hurst parameter as a measure of drug effect on the cardiovascular system. Estimates of parameters, which quantify heart rate dynamics are usually obtained from the time-domain or frequency-domain (Stein et al., 1994). Results obtained with these methods are confounded by the changing statistical properties of heartbeat IBI time-series. As shown in Fig. 1, time-domain measures such as the mean and standard deviation lose all phase information, while frequency-domain measures rely on assumptions of stationarity, i.e., that the means and standard deviations of the compared signals are equivalent. These criteria are not met with biologically derived physiological data such as the IBI. Measurement of cardiac signal dynamics using H does not require stationarity for the signals being compared, and H can thus be used to quantify drug effects under dynamic conditions as in the present study.

While the time-domain measures are all the same for the time-series presented in Fig. 1, the value of H changes as it reflects the magnitude and strength of the autocorrelation in the values of the time-series. H values approaching 0.5 from either extreme (0 or 1) are symptomatic of a breakdown in the long-range correlations of the heart IBI signal. These long-term correlations may represent an optimal level of feedback regulation between central and

peripheral determinants of heart rate (Peng et al., 1995). As a measure of the pharmacodynamic effect of agents which disrupt autonomic control of heart rate, determination of *H* may be complementary to measures of heart rate alone because of its apparent higher sensitivity to alterations in feedback regulation.

In summary, ketamine administration was found to induce robust decreases in *H*, and baseline cerebrospinal fluid 5-HIAA concentrations were inversely correlated with the magnitude of the ketamine response. The response to ketamine was also partially attenuated by ondansetron. We conclude that activation of 5-HT_3 receptors by ketamine is involved in modulation of cardiac signal dynamics, and that a stable trait associated with serotonin turnover influences the neural regulation of heart rate in the presence of ketamine.

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