Role of parasympathetic overactivity in water immersion stress-induced gastric mucosal lesion in rat

Yuan-Fang Xie, Qing Jiao, Shu Guo, Fu-Zhen Wang, Ji-Min Cao, and Zheng-Guo Zhang

Departments of 1Biomedical Engineering and 2Physiology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, School of Basic Medical Sciences, Peking Union Medical College, Beijing, China

Submitted 7 March 2005; accepted in final form 26 July 2005

STRESS ULCER IS A HIGHLY PREVALENT clinical complication. Fully understanding the mechanism of stress ulcer will increase our knowledge for the prevention and treatment of stress-related organ injury (25). Water immersion restraint stress (WRS) mimics the clinical acute gastric ulcerations caused by trauma, surgery, or sepsis (11) and has been widely accepted for studying stress ulcer (8, 17). It is known that autonomic nervous system (ANS), one of the main components of stress, exerts a profound influence on heart rate and digestion (10). However, the precise role of each component of the ANS in the gastric ulcer after stress exposure remains unclear as conflicting mechanistic explanations have been provided (2, 17, 35). Arakawa et al. (2) reported that excessive peripheral sympathetic activity plays an important role in the WRS model. In contrast however, Shichijo et al. (29) suggested the presence of a sympathetic hypofunction and a parasympathetic hyperfunction in the stomach contributing to the gastric lesion in the spontaneously hypertensive rats subjected to WRS. A recent report showed that vagal activity played an important part in the protection of gastric ulcer to WRS (7). Although controversial, it is generally believed that sympathovagal imbalance plays an important role in the gastric lesion in the rat WRS model (15, 29). Unfortunately, previous studies on WRS did not address in depth the issue of heart rate variability (HRV).

METHODS

Experimental protocol. All experimental protocols were approved by the Institutional Animal Care Committee of Peking Union Medical College. Fourteen male Wistar rats, weighting 250 ± 10 g, were fasted for 24 h but allowed free access to water before experimenta- tion. The rats were randomly divided into restraint control (RS) and WRS groups, seven in each group. The rats in both groups were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized by ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthetics were applied. The four limbs of each rat were anesthetized with ether inhalation at the beginning and then were kept conscious during the following experiments; no additional ether or other anesthesi...
before stress and every hour after the onset of stress. The RS group was similarly treated at a room temperature of 26–27°C, except not being immersed in the water. At the end of 5-h ECG recordings, the stomachs of all rats in both groups were isolated, opened along the major curvature, and inspected grossly and histologically by the hematoxylin and eosin (H-E) staining method. Digital photographs of the gastric mucosae were taken and then the gastric tissues were developed to prepare paraffin sections for H-E staining. To determine the causal relationship between parasympathetic activity and gastric mucosal lesion, an extra of seven male Wistar rats were subjected to bilateral cervical vagotomy 3 h before WRS. HRV analysis and gastric pathology were performed in these rats.

ECG recording and R wave detection. Surface ECGs were recorded with a computer-assisted PC-Lab system (Weixinsida Technology, Beijing, China) with a sample rate of 1,000 Hz. The R waves were detected off-line with a wavelet transform algorithm in MATLAB 6.5. The R-R interval time series was manually inspected for noise. Ectopic beats were linearly interpolated to exclude their influences on the HRV analysis.

Linear analysis of HRV. Mean R-R intervals, standard deviation of the normal-to-normal R-R intervals (SDNN) and root mean square of successive difference (RMSSD) of R-R intervals were used as time-domain parameters of HRV. These parameters were calculated by using the 5-min segments at 30-min intervals for the entire 5 h of observation. The mean value of these segments was expressed in milliseconds. Frequency domain analysis was based on power spectrum estimation, which was carried out with fast Fourier transform method. According to Berger’s algorithm (4), a continuous signal of a 51.2-s period was sampled at 20 Hz. The linear trend was removed from the R-R interval series, and a Hamming window was applied. The HRV spectral bands were defined as very low frequency (58.6–195.3 μHz), low frequency (LF, 195.4–605.4 μHz) and high frequency (HF, 605.5–2,500 Hz) (31). Three indexes were calculated: HF norm (the normalized area of the high-frequency component), LF norm (the normalized area of the low-frequency component), and LF-to-HF ratio (LF/HF). HF norm indicates vagal modulation on the cardiac pacemaker, whereas LF norm, especially LF/HF, is associated with cardiac sympathetic modulation (5, 30).

Poincaré plot analysis of R-R intervals. Poincaré plot is a diagram (scattergram) of each R-R interval plotted against the next. Poincaré plot analysis is a quantitative visual technique whereby the shape of the plot can be categorized into various functional classes, for example, the degree of heart failure in a subject (20). The plot provides summary information as well as detailed beat-to-beat information of the heart (19). The quantitative analysis of Poincaré plot is based on the differential temporal effects on the vagal and sympathetic tone in modulating beat-to-beat variability in heart rate. The analysis does not presume the existence of stationarity, as is the case of spectral analysis, nor does it require normal distribution of R-R intervals as is with standard deviation (20). In the present study, an ellipse was fitted to the Poincaré plot to quantitatively characterize beat-to-beat fluctuation. A set of axis oriented with the line of identity and its perpendicular is defined (32). The dispersion of the plot points is denoted with two measures, SD1 and SD2 (32). SD1 measures the width of the Poincaré cloud, indicating the dispersion perpendicular to the line of identity, therefore the level of short-term HRV (19, 20, 32). SD2 measures the length of the plot along the line of identity, representing the long-term HRV (19, 20, 32). SD1 is thought to be influenced by vagal efferent effect on the sinus node; the physiological meaning of SD2 is less well defined (32, 34).

Statistical analysis. All the data were expressed as means ± SE. Statistical significance of the differences between groups and among different stress phases were performed using Student’s two-tailed t-test for unpaired data. P values <0.05 were considered significant.

RESULTS

Gastric mucosal lesion induced by WRS and prevented by vagotomy. The gastric mucosae in rats subjected to RS alone remained intact, with no apparent lesions both in gross and histological inspections (Fig. 1, A and D). However, 5 h of WRS were associated with profound gastric mucosal lesion that was evident grossly and in histological sections in all WRS rats (Fig. 1, B and E). Serious mucosal hemorrhage, dotted ulcer, effusion, and structural collapse were clearly observed in H-E stains. The gastric mucosal lesion in the WRS group was limited to the phloum. Fundus was seldom affected, and duodenum remained intact in both groups. Bilateral cervical vagotomy almost totally prevented the gastric mucosal lesion

Fig. 1. Representative gross views and hematoxylin and eosin (H-E) stains of rat gastric mucosae. The mucosae were grossly intact in restraint stress (RS) (A) and Vagotomy + water immersion restraint stress (WRS) (C) groups but showed severe hemorrhage (B, black areas in the phloum) in the WRS group. H-E stains showed serious mucosal hemorrhage (brown areas in E), dotted ulcer, effusion, and structural collapse in WRS group (E) but no obvious mucosal lesion in RS (D) and Vagotomy+WRS groups (F). F, fundus; P, phloum; E, esophagus; D, duodenum.
induced by WRS, as shown both grossly (Fig. 1C) and histologically (Fig. 1F). Among the seven rats that underwent vagotomy and WRS, only one rat showed minimal gastric mucosal hemorrhage; the remaining six rats showed intact gastric mucosa.

Effects of WRS on linear parameters of HRV. Original recordings of ECGs and R wave detection marks are shown in Fig. 2. Heart rate decreased gradually with time in the WRS group (Fig. 2, left). In contrast, the heart rate slightly increased during the first 2 h and then kept a faster rate in the RS group (Fig. 2, right). The beat-to-beat variations of R-R intervals also increased with time in the WRS group but remained unchanged in the RS group.

Figure 3 showed the changes in the linear parameters of HRV in RS and WRS groups. These parameters did not show significant difference between the two groups before the onset of stress. The R-R intervals slightly but significantly decreased in the RS group during the experiment (Fig. 3A). However, the R-R intervals of WRS rats gradually increased with time after the onset of stress (117.6 ± 1.3 ms before stress; 165.4 ± 3.5 ms after 5 h of stress). The difference in the R-R intervals between the two groups was significant ($P < 0.001$). The SDNN and RMSSD significantly increased in the WRS group compared with the RS group (Fig. 3, B and C). These two parameters tended to decrease in the RS group but did not reach statistical significance at most time points (Fig. 3, B and C). The changes of SDNN and RMSSD in the WRS group showed three phases: a rapid rising phase (0–2 h), a plateau phase (2–4 h), and a declining phase (4–5 h). The differences of SDNN and RMSSD at each of these three phases were

Fig. 2. Examples of original ECG recording in both groups. Vertical bars are R-wave detection marks. The figure shows that heart rate decreased gradually in the WRS group, but no significant change was observed in the RS group. The numbers are the respective R-R intervals. Note that there is a higher R-R interval variation 5 h after WRS and a lower R-R interval variation 5 h after RS.
statistically significant ($P < 0.05$ or $< 0.001$) between the RS and WRS groups (Fig. 3, B and C).

LF norm and LF/HF increased at the first hour of stress and then decreased gradually to the baseline (before stress) value or even lower in RS and WRS groups (Fig. 3, D and F). However, the trends of the two parameters were similar and did not show significant statistical difference between the two groups (Fig. 3, D and F). In the WRS group, HF norm did not change during the first hour of stress but increased to peak in 1–3 h and then declined slightly at 4–5 h of stress (Fig. 3E). HF norm decreased during the first 2 h ($P < 0.001$) and then increased in RS group ($P < 0.05$) (Fig. 3E). However, this increase in HF norm in RS group was significantly less than WRS group ($P < 0.001$) (Fig. 3E).

For a quantitative beat-to-beat analysis of the Poincaré plots, SD1 and SD2 did not show significant differences between RS and WRS groups before stress (Fig. 5). SD1 showed a similar trend as RMSSD in both groups: it increased significantly during the first 2 h of stress and then remained at a higher level in the WRS group (Fig. 5A). In the RS group, however, SD1 decreased slightly but significantly during the first hour of stress, kept a lower value in the following 3 h, and then increased slightly in the last hour of stress (Fig. 5A). SD1 showed a significant increase in the WRS group compared with the RS group (Fig. 5A). The changes of SD2 showed a similar pattern as SDNN in both RS and WRS groups (Fig. 5B). SD2 showed significant differences between the two groups only after 2 h of stress (Fig. 5B).

**DISCUSSION**

The main observations of the present study are 1) WRS induced serious gastric mucosal lesion; 2) parasympathetic overactivity is the predominant autonomic response in the WRS model; 3) bilateral cervical vagotomy prevented the gastric mucosal lesion induced by WRS; and 4) RS alone induced only a mild sympathetic hyperactivity and failed to induce gastric mucosal lesion. These results suggest a causal relationship between parasympathetic overactivity and gastric mucosal lesion in rat WRS model.

The direction and intensity of autonomic responses appear to be stress specific. Stressors such as mental challenge (9), light-induced arousal (31), and standard psychological reflection (6) are all correlated with an increase in sympathetic tone, a decrease in parasympathetic tone, or both. In the case of the forehead cold pressor stress (18), long period of Antarctica
residence (12), and water immersion stress (17), a decrease of the sympathetic component and an increase of parasympathetic component were observed. Therefore, it is necessary to clarify the nature of stress to provide rational strategies for the treatment of stress injuries.

The control. In the present study, we used restrained rats as the control instead of free, unrestrained rats. The purpose of this choice was to remove the possible influence of restraint stress on the analysis of water immersion stress. Furthermore, “restraint” would facilitate a stable ECG recording without impairing the analysis.

Autonomic responses to RS. The present study suggests that rats subjected to RS alone are in moderate sympathetic dominance and parasympathetic withdrawal, especially during the first 2 h of stress, as shown by decreases in mean R-R intervals, RMSSD, and HF norm, and increases in LF and LF/HF. SDNN did not show significant change during the whole course of RS, as SDNN reflects the overall R-R interval fluctuations (3). These results are in accord with the reports (21, 28) that RS alone stimulated the sympathetic nerve, especially during the early stage (1 or 2 h after the onset of stress). The significant decrease (Fig. 5B) in SD1 and SD2 in RS group also suggests a lower fluctuation of R-R intervals and thus a hyperactivity of sympathetic tone.

Parasympathetic dominance in WRS. The HRV analyses of the present study demonstrate that parasympathetic overactivity predominated during the WRS, as shown by significant increases in mean R-R intervals, SDNN, RMSSD, HF norm, and SD1. These HRV parameter variations are similar with that of human water immersion (27).

LF norm and LF/HF, parameters reflecting sympathetic tone and/or sympathovagal balance, increased in the early phase (first hour) of stress and then decreased during the subsequent period of stress and did not show significant differences between the two groups, suggesting a moderate, if not equal, increase in sympathetic tone in the initial phase of stress in...
both RS and WRS groups. After the initial period of stress, parasympathetic activity in the WRS group became dominant, whereas no such parasympathetic dominance was observed in the RS group. SDNN, which reflects the overall autonomic activity and fluctuations of heart rate (3), increased significantly in the WRS group compared with the RS group. This result also suggests an increase in parasympathetic tone in the WRS group.

Poincaré plotting gives visually distinguishable patterns of heart rate fluctuations and is a useful tool to describe the autonomic effect on heart rate dynamics. In the quantitative analysis of Poincaré plots (Fig. 5A), SD1 characterizes the beat-to-beat variation of HRV (13). Instantaneous beat-to-beat changes in the R-R intervals are mediated by vagal efferent activity, because vagal effects on the sinus node are known to develop faster than sympathetically mediated effects (14, 32). Furthermore, the changes in SD1 paralleled the RMSSD. Thus SD1 could be considered as a nonlinear indicator of parasympathetic activity as suggested by Mourot et al. (24) and Lund et al. (22). The present study also showed that SD1 increased significantly 1 h after the onset of WRS but decreased significantly at the same phase in the RS group. This phenomenon indicates a vagotonia in WRS group.

Taken together, the present study suggests that WRS-loaded rats manifest autonomic imbalance characterized by increased vagal tone. This is consistent with previous studies using circulatory stress hormones and R-R intervals as indexes of altered autonomic tone (17). Using the HRV approach, we found in the present study that WRS stress model is associated with parasympathetic overactivity.

**Time course of the sympathovagal balance in WRS and RS.**

Rats subjected to WRS appeared to show a slight increase in sympathetic tone during the early phase of stress, as indicated by the increases in both LF norm and LF/HF. However, the increases in these two parameters did not reach a statistical significance. Moreover, SD2 showed no significant difference in the first hour between RS and WRS groups, suggesting similar levels of sympathetic tone during the early phase of stress in the two groups (32). Both the present study and the reports by others (16, 17) suggest that excitation of the sympathetic nerves occurs at the beginning of the WRS loading, followed by parasympathetic overactivity. In RS rats, the autonomic activities showed a transient and moderate sympathetic overactivity.

In the present study, we found certain inconsistencies between the heart rate and HF norm, RMSSD, and SD1. For example, 4 h after water immersion, heart rate continued to slow down, but HF norm, RMSSD, and SD1 began to decrease instead of increase. This phenomenon could not be the result of slow deep respiration as reported by Schipke and Pelzer (27), because no increase in the LF range was observed (27). Moreover, the structures of the Poincaré plots became obvious condensed after 4-h stress in WRS group, which may suggest a depressed variability in heart rate dynamics possibly resulting from self-adaptive regulation of the ANS (17). Similar phenomena between automatic activity and heart rate were also reported by Ahmed et al. (1) and Yutaka et al. (36). These inconsistencies might be a result of the saturating effect as suggested by Malik and Camm (23). An increase of R-R intervals does not necessarily parallel with an increase of R-R interval fluctuation if parasympathetic tone is saturated by long-term overstimulation.

**Mechanisms of gastric mucosal lesion in WRS.** Kitagawa et al. (21) reported that rat gastric acid output was increased remarkably by WRS, and this increase lasted while the stress was present, yet the mucosal blood flow did not show a corresponding increase. The stress-induced increase in acid output correlated well with the severity of mucosal lesions. In the present study, bilateral cervical vagotomy prevented the gastric mucosal lesion in the WRS model. On the basis of these findings, we suggest that WRS-induced gastric mucosal lesion is basically a digestive ulcer resulted from parasympathetic overactivity and related increase in acid output at the basis of reduced mucosal resistance due potentially to mucosal ischemia and oxidative stress injury.

**Arrhythmias in the WRS and RS models.** Few (<2%) ectopic beats were found in both WRS and RS groups. The occurrences of ectopic activities appeared to be higher in the WRS group than the RS group but did not show statistical significance between the two groups. Atrial fibrillation was not found in all animals. Thus the occurrence of arrhythmias in this study did not appear to provide mechanistic implications and did not impair the efficiency of HRV analysis.
Limitations. The pathophysiology of WRS is complicated; many factors or signal pathways are more or less involved in the ulceration; for example, the effects of cardiac and pulmonary stretch receptor stimuli by water immersion on autonomic output, the influence of psychological stress on gastric mucosal perfusion, the effects of mucosal oxidative stress and inflammation on mucosal stability, and so on. In this study, we investigated only the relationship between sympathovagal imbalance and gastric mucosal lesion by HRV analysis. A further investigation of other factors may offer deeper insight into the mechanisms of WRS-induced gastric mucosal lesion. In conclusion, parasympathetic overactivity is the predominant response of the ANS to WRS and is possibly the leading cause of WRS-induced gastric mucosal lesion. RS induces transient and mild sympathetic hyperactivity and is not the cause of gastric ulcer in this model.

ACKNOWLEDGMENTS

We thank Drs. Li-Hui Huang, Xiao-Lin Yang, and Wei Hao for assistance. Ji-Min Cao and Zheng-Guo Zhang are both corresponding authors in this work.

GRANTS

This study was supported by grants from the National Natural Science Foundation of China (30370394 to Z.-G. Zhang; 30125016, 30313902 and 30370565 to J.-M. Cao).

REFERENCES