Journal of Stress Physiology & Biochemistry, Vol. 7 No. 2 2011, pp. 164-174 ISSN 1997-0838

ORIGINAL ARTICLE

PHYSICAL AND PSYCHOLOGICAL STRESS HAVE SIMILAR EFFECTS ON GASTRIC ACID AND PEPSIN SECRETIONS IN RAT

Nabavizadeh[1] Fatemeh, Mohammad Vahedian[2], Hedayat Sahraei[3], Soheila Adeli[1], and Ehsan Salimi[1]

[1] *Department of physiology, Tehran University of Medical Sciences, Tehran, Iran*
[2] *Department of Orthopaedy, Tehran University of Medical Sciences, Tehran, Iran*
[3] *Applied Neuroscience Research Center, Baqyiatallah (a.s) University of Medical Sciences, Tehran, Iran*

E-mail: **Nabavizadeh2000@yahoo.com**

Phone: 00989133410451
Fax: 00982166419484

Received April 23, 2011

Stress is one of the most important health and social problems. Previous studies have demonstrated stress influence on the clinical course of a number of gastrointestinal diseases, but its physical and psychological effects on gastric acid and pepsin secretions are largely unknown.

48 male wistar rats weighing 200-250 gr were used in this study. Animals were divided into 6 groups (n=8); Control, Physical stress**,** Psychological stress, L-NAME+ Physical stress and L-NAME+ Psychological stress groups. In this study**,** electrical shock generated in a communication box was used as physical shock and the emotional stress was as psychological stress.

Gastric juice was collected by washout technique. Acid output was also measured by digital titrator. Gastric pepsin and nitric oxide (NO) metabolites were quantified using Anson and Griess micro assay methods respectively.

Basal and stimulated gastric acid and pepsin in physical and psychological stress groups were significantly more than others. NO metabolites level of gastric tissue in physical and psychological stress groups (286.9 ± 5.8, 287.7 ± 5.7 µmol/gr weight wet tissue, respectively) were significantly more than other groups. But no significant differences among basal and stimulated gastric acid, pepsin and NO metabolites level were seen in physical and psychological stress groups.

Key words: Physical / Psychological / Stress / Gastric acid / Pepsin / Rat

ORIGINAL ARTICLE

PHYSICAL AND PSYCHOLOGICAL STRESS HAVE SIMILAR EFFECTS ON GASTRIC ACID AND PEPSIN SECRETIONS IN RAT

Nabavizadeh[1] Fatemeh, Mohammad Vahedian[2], Hedayat Sahraei[3], Soheila Adeli[1], and Ehsan Salimi[1]

[1] *Department of physiology, Tehran University of Medical Sciences, Tehran, Iran*
[2] *Department of Orthopaedy, Tehran University of Medical Sciences, Tehran, Iran*
[3] *Applied Neuroscience Research Center, Baqyiatallah (a.s) University of Medical Sciences, Tehran, Iran*

E-mail: **Nabavizadeh2000@yahoo.com**

Phone: 00989133410451
Fax: 00982166419484

Received April 23, 2011

Stress is one of the most important health and social problems. Previous studies have demonstrated stress influence on the clinical course of a number of gastrointestinal diseases, but its physical and psychological effects on gastric acid and pepsin secretions are largely unknown.

48 male wistar rats weighing 200-250 gr were used in this study. Animals were divided into 6 groups (n=8); Control, Physical stress**,** Psychological stress, L-NAME+ Physical stress and L-NAME+ Psychological stress groups. In this study**,** electrical shock generated in a communication box was used as physical shock and the emotional stress was as psychological stress.

Gastric juice was collected by washout technique. Acid output was also measured by digital titrator. Gastric pepsin and nitric oxide (NO) metabolites were quantified using Anson and Griess micro assay methods respectively.

Basal and stimulated gastric acid and pepsin in physical and psychological stress groups were significantly more than others. NO metabolites level of gastric tissue in physical and psychological stress groups (286.9 ± 5.8, 287.7 ± 5.7 µmol/gr weight wet tissue, respectively) were significantly more than other groups. But no significant differences among basal and stimulated gastric acid, pepsin and NO metabolites level were seen in physical and psychological stress groups.

Key words: Physical / Psychological / Stress / Gastric acid / Pepsin / Rat

Stress is a term in psychology and biology, first coined in the 1930s, which has become a commonplace of popular parlance in more recent decades. It refers to the consequence of the failure of an organism- human or animal to respond appropriately to emotional or physical threats,

whether actual or imagined (Kennard, 2008).

Stress is one of the most important health and social problems. Nowadays, human are exposed to many physical and psychological stress such as death of children, wife or husband, war, earthquake, torrent and so on. These are responsible for various pathological conditions such as cardiovascular, hormonal and gastrointestinal diseases (Schellenbaum et al., Swain et al., 2000). Stress has also been implicated as a cofactor in the severity and progression of a number of ailments (Eskiocak et al., 2006).

Stress symptoms commonly include a state of alarm and adrenaline production, short-term resistance as a coping mechanism, and exhaustion. Irritability, muscular tension, inability to concentrate and a variety of physiological reactions such as headache, elevated heart rate, inability to relax, feeling lonely, isolated or depressed, aches and pains, diarrhea or constipation, nausea, dizziness, chest pain, and increased alcohol and nicotine consumption are also mentioned in the literature (Barclay et al., 2005; Seyle, 1950; Glavas et al., 2007).

The autonomic nervous system provides the rapid response to stress commonly known as the fight-or-flight response, engaging the sympathetic nervous system and withdrawing the parasympathetic nervous system (Tsigos et al., 2002).

Previous studies have demonstrated stress influence on the clinical course of a number of gastrointestinal diseases (Monnikes et al., 2001; Gue et al., 1987), but the effects of physical and psychological stress on gastric acid and pepsin and the underlying mechanisms are largely unknown. Therefore, the purpose of this study was to investigate the effects of physical and psychological stress on gastric acid and pepsin and its possible mechanisms (nitric oxide role) in rats.

MATERIALS AND METHODS

The procedure was in accordance with the guidelines for the care and use of laboratory animals of Tehran University of medical Sciences, Tehran, Iran. Locally produced male wistar rats weighing 200-250 gr were used. They maintained in a temperature controlled environment on 12: 12 hour light: dark cycle with free access to food and water. Animals were classified into the following 6 groups (n=8):

1- Physical stress: Animals were kept in the stress box. They were received 10 second electrical shock and 50 second rest for 1 hour on a daily basis for 2 weeks.

2- Psychological stress, Animals were put in the stress box for 1 hour each day and exposed to psychological shock for 2 weeks.

3- Control, Put in the stress box for 1 hour each day without receiving any form of mentioned stress for the same period.

4- Physical stress + L-NAME: Daily pretreated of L-NAME (40 mg/kg, ip) 5 minutes before physical stress for the same period (Nishida et al., 1997).

5- Psychological stress+ L-NAME: Daily pretreated of L-NAME (40 mg/kg, ip) 5 minutes before psychological stress for the same period (Nishida et al., 1997).

6- Control+ L-NAME: Daily pretreated of L-NAME (40 mg/kg, ip) for the same period

Stress stimulation with the communication box

The communication box was designed appropriate for the emotional stress paradigm in this study. It consists of 8 compartments (16 Ч 16 cm)

separated by transparent plastic boards. The boards have a few pores to allow animals to receive visual, auditory and olfactory sensation cues from the neighboring animal, but prevent them from physical contact. Each compartment was equipped with a grid floor made up of 5 mm diameter stainless steel rods placed 1.3 cm apart each other (Vincent et al., 2002). An electric generator (current of 1 MA) (Borj Sanat Co.Iran, sensitivity 0.1-10 MA) was connected to the grid floor to generate an electric foot shock for 10 seconds with an interval of 50 second. Four compartments of the grid floor were insulated by plastic plates to prevent electric foot shock and served as non-foot-shock compartments for the emotional stress rats.

Surgical procedure and measurement of gastric acid and pepsin secretions

Main experiment was started after stress induction,. Before each experiment, animals were deprived of food for 24 hr with a free access to water. Under general anesthesia (Thiopental sodium 50 mg/kg, ip) tracheotomy and laparotomy was performed (Nabavizadeh et al., 2003). 30 minutes was left for a recovery time and then gastric juice was collected by washout technique (Nabavizadeh et al., 2003). For measuring basal acid output, 1 ml normal saline was entered into the stomach, another 1 ml was added15 minutes later. Then stomach content was aspirated for acid titration using digital titrator (Basic Titrino, Metrohm, 794) by 0.001 N NaOH. The remainder gastric juice (1 ml) was used for pepsin measurement by Anson method(Nabavizadeh et al., 2002). In order to measure stimulated acid and pepsin secretion, pentagastrin (25 µg/kg, ip) was used (Kato et al., 1998). Stimulated acid and pepsin secretions were measured after 15 minutes. Experiments started at 8 AM everyday to omit circadian rhythm effect

Corticostrone level was measured by ELISA method. Gastric and proximal duodenum tissue was removed and kept in fixative solution (formalin 10%) for histological study. Gastric tissue nitric oxide (NO) metabolites were also measured using Griess micro assay method (Nabavizadeh et al., 2009).

Data were expressed as Mean ± SEM. Analysis of variance (ANOVA) followed by Tukey post test was used for statistical analysis. $P< 0.05$ was considered to be statistically significant.

RESULTS

There were no significant differences in serum corticostrone between control and control + LNAME groups (255.73 ± 8.2, 265.22 ± 7.9 ng/ml respectively) (Fig. 1). But serum corticostrone level was significantly more in physical stress, psychological stress, physical stress + LNAME and psychological stress + LNAME (590.1 ± 9.8, 586.12 ± 9.42, 582.2 ± 8.8 and 580 ± 9.2 ng/ml) groups than in control and control + LNAME ($P<0.001$) (Figure 1).

The mean basal and stimulated gastric acid secretion in physical stress (2.01 ± 0.3 basal, 4.46 ± 0.2nmol/ml stimulated) and psychological stress (1.98 ± 0.2 basal, 4.28 ± 0.3 nmol/ml stimulated) groups were significantly more than in control , control + LNAME, physical stress + LNAME, and psychological stress + LNAME groups ($P<0.001$) (Table 1). But there were no significant differences between basal and stimulated acid secretion in physical stress and psychological stress groups (Table 1). No significant changes were also seen between basal and stimulated acid secretion in control, control + LNAME, physical stress + LNAME and psychological stress + LNAME groups (Table 1).

The mean basal and stimulated gastric pepsin secretion in physical stress (2.92 ± 0.3 basal, 3.88 ± 0.3 µg/ml stimulated) and psychological stress (2.88 ± 0.4 basal, 3.68 ± 0.4 µg/ml stimulated) groups were significantly more in control, control + LNAME, physical stress + LNAME and psychological stress + LNAME ($P<0.05$) (Table 2). But there were no significant differences between basal and stimulated pepsin secretion in physical and psychological stress groups (Table 2). Also, there were no significant differences between basal and stimulated pepsin secretions in control, control + LNAME, physical stress + LNAME and psychological stress + LNAME groups (Table 2).

Table 1: The effect of physical and psychological stress on basal and stimulated acid secretions (Mean ± SE, n=8)

States / Groups	Basal acid output (mmol/ml/15min)	Stimulated acid output (mmol/ml/15min)
Control	0.4±0.02	0.92±0.04
Control+LNAME	0.3±0.03	0.72±0.02
Physical stress+LNAME	0.46±0.02	0.8±0.03
Psychological stress+LNAME	0.38±0.04	0.78±0.03
Physical stress	2.01±0.3**	4.46±0.2**
Psychological stress	1.98±0.2**	4.28±0.3**

**P<0.001 comparison of physical and psychological stress groups with other in basal and stimulated acid secretions

Table 2: The effect of physical and psychological stress on basal and stimulated pepsin secretions (Mean ± SE, n=8)

States / Groups	Basal pepsin output (µg/ml/15min)	Stimulated pepsin output (µg/ml/15min)
Control	1.38±0.4	2.74±0.4
Control+LNAME	1.25±0.3	2.75±0.2
Physical stress+LNAME	1.58±0.3	2.94±0.3
Psychological stress+LNAME	1.6±0.2	2.9±0.22
Physical stress	2.92±0.3*	3.88±0.3*
Psychological stress	2.88±0.4*	3.68±0.4*

*P<0.05 comparison of physical and psychological stress groups with other in basal and stimulated pepsin secretions

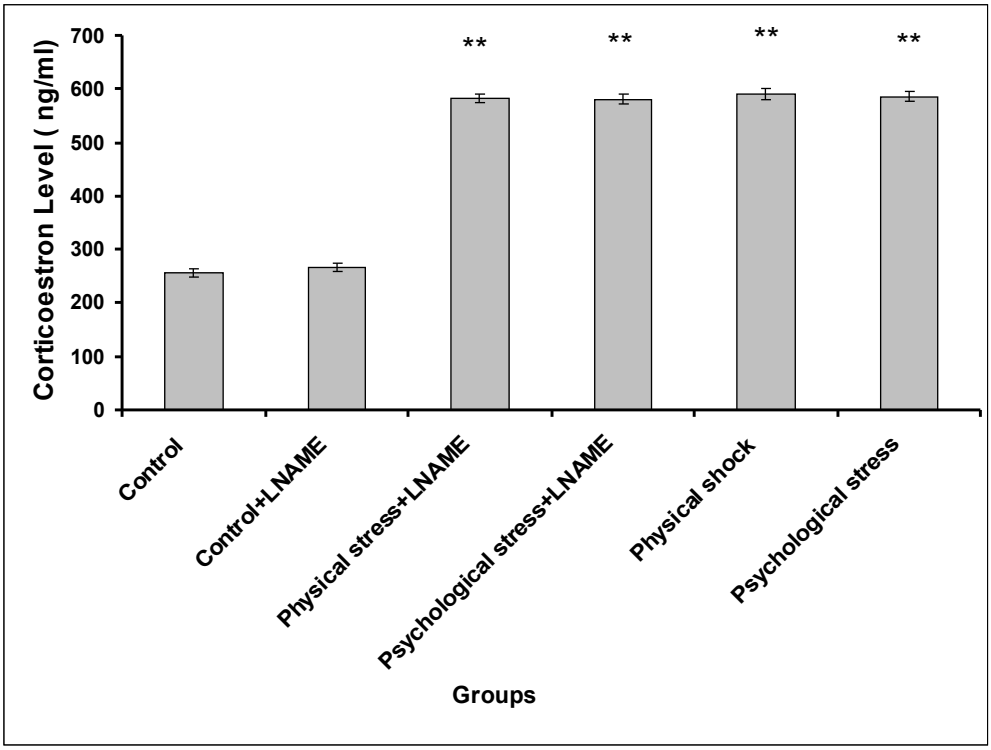

Figure 1 The level of corticoestron in physical, psychological stress groups and others (Mean ± SE, n=8)
**P<0.001 Comparison of physical, psychological, physical stress + LNAME, psychological stress + LNAME groups with control and control + LNAME groups in the corticoestron level.

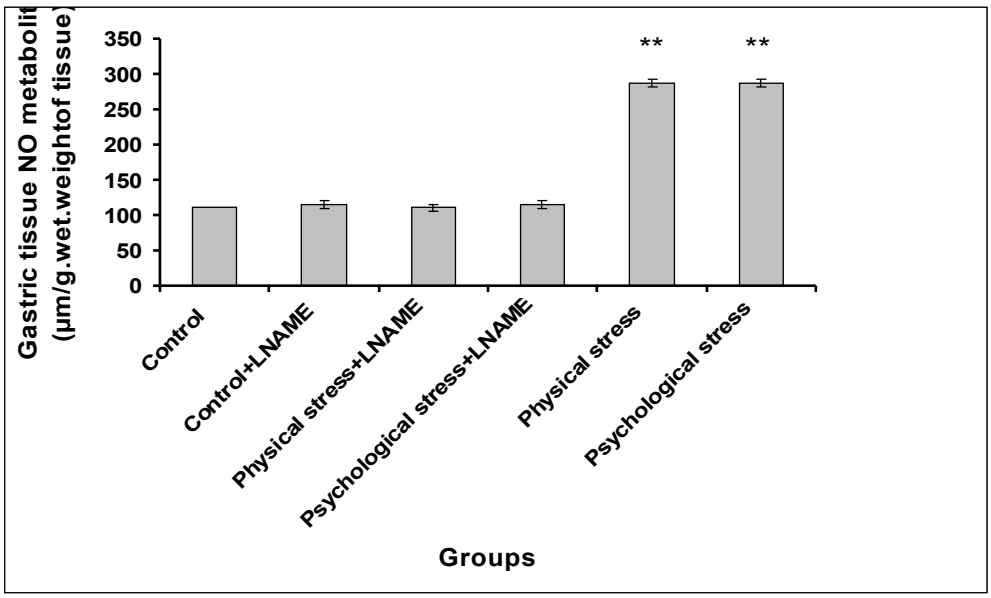

Figure 2 The level of gastric tissue NO metabolites in physical, psychological stress groups and others (Mean ± SE, n=8)
**P<0.001 Comparison of physical, psychological stress groups with physical stress + LNAME, psychological stress + LNAME , control and control + LNAME groups in the gastric tissue NO metabolites level.

Table 3: Histological findings of gastric and proximal duodenum in the physical stress, psychological stress , physical stress + LNAME, psychological stress + LNAME , control and control + LNAME groups (n=8)

Histological changes / Groups	Gastric mucosal necrosis	Proximal duodenal mucosal necrosis	Gastric mucosal necrosis + Proximal duodenal mucosal necrosis	Chronic gastritis	Normal
Control	_	_		_	8
Control+LNAME	_	_		_	8
Physical stress+LNAME	_	_		_	8
Psychological stress+LNAME	_	_		_	8
Physical stress	_	2	4	2	_
Psychological stress	_	_	4	4	

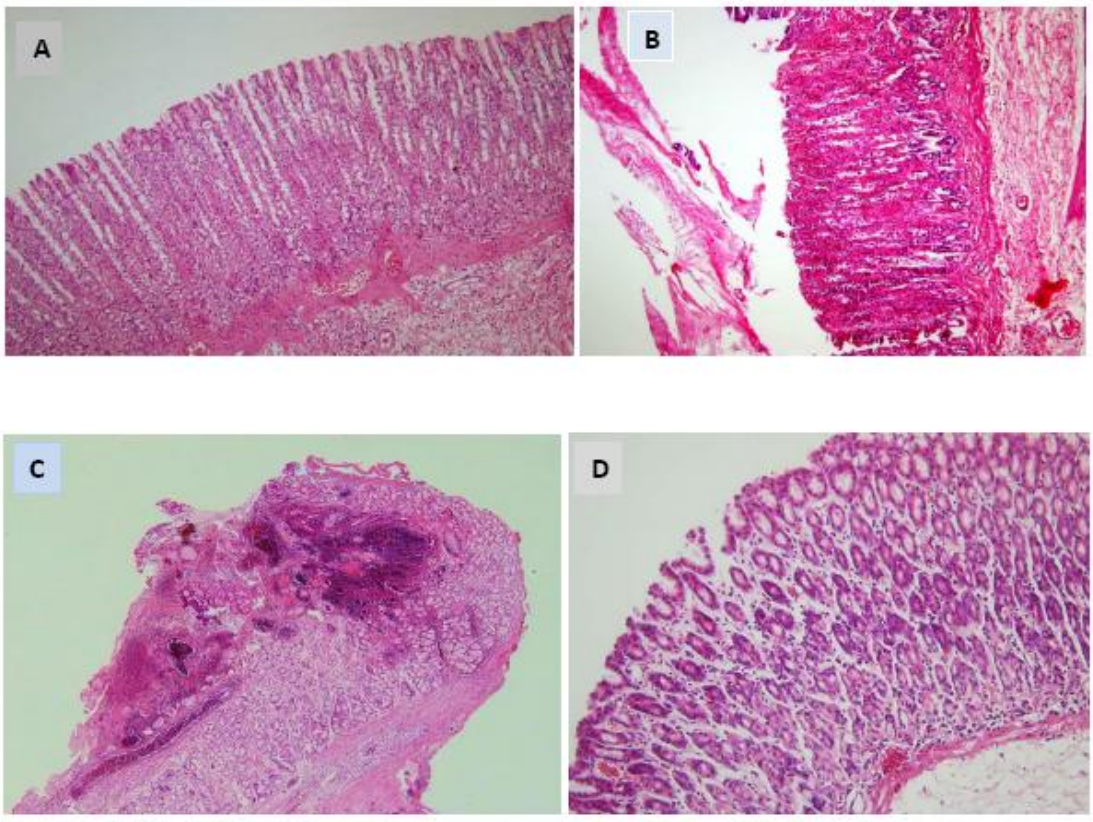

Figure 3: Histological findings of gastric and proximal duodenum in the physical stress, psychological stress , physical stress + LNAME, psychological stress + LNAME , control and control + LNAME groups
A: Normal gastric mucosal
B: Gastric mucosal necrosis
C: Duodenal mucosal necrosis (the mucosa is totally necrotic and hemorrhagic)
D: Chronic gastritis

Figure 2 shows that the level of NO metabolites of gastric tissue in physical and psychological stress groups (286.9 ± 5.8, 287.7 ± 5.7 µmol/gr weight wet tissue, respectively) were significantly more than control, control + LNAME, physical stress + LNAME and psychological stress + LNAME (119.5 ± 5.2, 115 ± 4.8, 110.2 ± 5.1 and 114.5 ± 4.8 µmol/gr weight wet tissue, respectively) ($P<0.001$). But there were no significant differences between level of NO metabolites of gastric tissue in physical and psychological stress groups (Figure 2). Also, there were no significant differences between amounts of NO metabolites of gastric tissue in control, control + LNAME, physical stress + LNAME and psychological stress + LNAME groups (Figure 2).

Histological study showed, that gastric tissues were normal in control, control + LNAME, physical stress + LNAME and psychological stress + LNAME groups (Table 3, Figure 3A).While gastric tissues were abnormal (gastric necrosis, duodenal necrosis and gastritis) in physical and psychological stress groups (Table 3, Figure 3B, 3C, 3D, respectively).

DISCUSSION

In this study, serum corticostrone level was significantly increased in all stress groups i.e physical stress, psychological stress, physical stress + LNAME and psychological stress + LNAME in comparison to control and control + LNAME groups. Article reviewshows the same result in which an increase in corticosteroids especially cortocostrone has been showed in rat induced physical & psychological stress (. Stockham et al., 1964; Baig et al., 2006; Min et al., 2010).

Mean basal and stimulated gastric acid and pepsin secretions were also significantly increased in physical and psychological stress groups compared with control, control + LNAME, physical stress + LNAME and psychological stress + LNAME groups. There were no significant differences between basal and stimulated acid and pepsin secretions in physical and psychological stress groups that means physical and psychological stress have similar effects on gastric acid and pepsin secretions. In this study, NO metabolites of gastric tissue has rather been unchanged in the physical and psychological stress groups, but their levels were significantly more in physical and psychological stress groups than others``.

The hypothalamic - pituitary-adrenal axis (HPA) is a major part of the neuroendocrine system which interacts with the hypothalamus, pituitary and the adrenal glands and mediates adrenocorticotropic hormone (ACTH) release from the pituitary into the bloodstream and finally causes cortisol secretion and other glucocorticoids from the adrenal cortex (Tsigos et al., 2002).

Previous study was shown that stress could increase corticosteroids, and they in return stimulated gastric acid secretion in rat (Stockham et al., 1964). Previous researchers have also shown psychological stress-induced enhancement of brain lipid peroxidation via nitric oxide (NO) system in mice (Matsumoto et al., 1999). According to Eskiocak et al, psychological stress can increase sperm motility and semen quality through arginine-NO pathway and NO synthase activation (Eskiocak et al., 2006).

Schoen and coworkers showed that hemodynamic changes could increase shear stress and No release, followed by liver regeneration cascade triggering (Schoen et al., 2001). Physical (mechanical) stress can also elicit nitric oxide (NO) formation and DNA fragmentation in Arabidopsis

thaliana plant (Garces et al., 2001).

In our study, NO metabolites level of gastric tissue in physical and psychological stress animals were significantly more than other groups, that means both physical and psychological stress may increase gastric acid and pepsin secretion via NO pathway (Matsumoto et al., 1999; Eskiocak et al., 2006; Schoen et al., 2001). Animal treatment with LNAME before being exposed to physical and psychological stress resulted in gastric tissue NO, acid and pepsin level similar to the control group which implies possible NO mediatory role on gastric secretion in physical and psychological stress conditions. Other studies confirmed NO-synthase presence in gastric epithelial cells and stimulatory effect of NO donors on gastric mucus secretions. (Brown et al., 1993).

Pique et al depicted endogenous NO contribution in gastric mucosal vasodilation and acid secretion (Pique et al., 1992). NO has been shown to have a dual role on gastric acid secretion, i.e in some studies it has a decremental while in others it has an incremental effect on gastric acid secretions via stimulating histamine release from ECL cells (Kato et al., 1998; Barrachina et al., 1994). Vagal stimulation or pentagastrin has also been shown to have stimulatory effect on acid production via NO release (Hasebe et al., 2005; Hasebe et al., 2001).

In our study, physical and psychological stress has increased gastric acid and pepsin secretion possibly through NO release, based on the obvious similarity between the gastric tissue NO and gastric acid and pepsin amount in control and LNAME + stress groups. Although, many mechanisms may be involved in stress induced gastric secretions, our findings strongly recommend possible NO role here.

Our histological studies showed either necrosis or chronic inflammation in gastric and duodenal tissue of animals under stress or psychological stress, but, these tissues were almost normal in control, control + LNAME, physical stress + LNAME and psychological stress + LNAME groups.

It is note-worthy that all the changes in acid and pepsin secretions, gastric tissue NO metabolites level, and gastric and duodenal histology were similar in physical and psychological stress groups.

CONCLUSION

This study show that physical and psychological stress increase gastric acid & pepsin secretions possibly by raising the gastric tissue NO level. Increased gastric acid & pepsin secretions in return cause necrotic and inflammatory changes of gastric and duodenal tissue. It is note-worthy that these changes are similar in physical and psychological stress groups.

REFERENCES

Baig A, Siddiqui I, Naqvi H, Sabir S, Jabbar J, Shahid M.(2006). Correlation of serum cortisol levels and stress among medical doctors working in emergency departments. *J. Coll Physicians Surg Pak*. **16(9)**: 576-580.

Barrachina D, Calatayud S, Esplugues J, Whittle, Moncada S. **(**1994). Nitric oxide donors preferentially inhibit neurally mediated rat gastric acid secretion. *Eur. J. Pharmacol*. **262**: 181-183.

Barclay L, Vega C. (2005). Emotional stress may precipitate severe, reversible left ventricular dysfunction. *N Eng J Med*. **352**: 539-548.

Brown J F, Keates A C, Hanson P J, Whittle B J. (1993). Nitric oxide generators and c GMP stimulate mucus secretion by rat gastric mucosal cells *AJP- Gastrointestinal and Liver*

Physiology **265**: G418-G422.

Eskiocak S, Gozen AS, Taskiran A, Kilic AS, Eskiocak M and Gulen S.(2006). Effect of psychological stress on the L-arginine-nitric oxide pathway and semen quality. Brazilian *Journal of Medical and Biological Research*. **39**: 581-588.

Garces H, Durzan D, Pedroso M C. (2001). Mechanical stress elicits nitric oxide formation and DNA fragmentation in Arabidopsis thaliana. *Annals of Botany*. **87**: 567-574.

Glavas M M, Weinberg J.(2007). Stress, Alcohol Consumption, and the Hypothalamic-Pituitary-Adrenal Axis. *Nutrition and Health Nutrients, Stress, and Medical Disorders*. **17**: 165-183.

Gue Michиle , Fioramonti Jean , Frexinos Jacques, Alvinerie M, Bueno Lionel. (1987**).** Influence of acoustic stress by noise on gastrointestinal motility in dogs. *Digestive Diseases and Sciences*. **32(12)**: 1411-1417.

Hasebe K, Horie S, Komasaka M, Yano S, Watanabe K. (2001). Stimulatory effects of nitric oxide donors on gastric acid secretion in isolated mouse stomach. *Eur. J. Pharmacol*. **420**: 159-164.

Hasebe K, Horie S, Noji T, Watanabe K, Yano S. (2005). Stimulatory effects of endogenous and exogenous nitric oxide on gastric acid secretion in anesthetized rats. *Nitric Oxide*. **13**: 264-271.

Kato S, Kitamura M, Korolkiewicz RP, Takeuchi K. (1998). Role of nitric oxide in regulation of gastric acid secretion in rats effects of no donors and no synthase inhibitor. *Br J pharmacol.* **23**: 839-846

Kennard J**.** A Brief History of the term Stress**.** **(**2008). Retrieved from http://www.healthcentral.com/anxiety/c/1950/30437/history-term-stress

Matsumoto K, Yobimoto K, Houng N T, Abdel-Fattah M, Hien T V, Watanabe H. (1999). Psychological stress-induced enhancement of brain lipid peroxidation via nitric oxide systems and its modulation by anxiolytic and anxiogenic drugs in mice. *Brain Research* **839**: 74-84.

Min L Z, Wang L X, Jiang L C, Zhu J X, Geng F Y, Qiang F. (2010). Relationship between plasma cortisol levels and stress ulcer following acute and severe head injury. *Med Princ Pract*. **19**: 17-21.

Mцnnikes H, Tebbe J J, Hildebrandt M, Arck P, Osmanoglou E, Rose M, Klapp B, Wiedenmann B, Heymann-Mцnnikes I.(2001). Role of Stress in Functional Gastrointestinal Disorders Evidence for Stress-Induced Alterations in Gastrointestinal Motility and Sensitivity. *Dig Dis* **19**: 201-221

Nabavizadeh F, Salimi E, Sadroleslami Z, Vahedian J**.** Saffron (Crocus sativus) increases gastric acid and pepsin secretions in rats: Role of nitric oxide (NO). *Afr. J. Pharmacy and Pharmacol.* 2009; **3(5)**: 181-184

Nabavizadeh F , Zahedi Asl S , Garib Naseri M K, Vahedian J. (2003). Effects of thyroid hormones on basal and stimulated gastric acid secretion due to histamine, carbachol and pentagastrin in rats. *Saudi Med J.* **24(4)**: 341-346.

Nabavizadeh F , Zahedi Asl S , Garib Naseri M K, Vahedian J. (2002). Effect of thyroid hormones on distension induced gastric acid and pepsin secretions in rats . *Annals of Saudi Medicine* . **22(5-6)**; 308-311.

Nishida K, Ohta Y and Ishiguro I. (1997). Role of

gastric mucosal constitutive and inducible nitric oxide synthases in the development of stress-induced gastric mucosal lesion in rats. *Biochem Biophys Res Commu*n. **18(236)**: 275-279.

Pique J M, Esplugues J V, Whittle B J. (1992). Endogenous nitric oxide as a mediator of gastric mucosal vasodilatation during acid secretion. *Gastroenterology*. **102(1)**: 168-174.

Schellenbaum GD**,** Rea TD and Smith NL. (2002). Mental stress-induced ischemia and all-cause mortality in patients with coronary artery disease. *Circulation*, **106**: 183-184.

Schoen J M, Wang H H, Minuk G Y, Lautt W W. (2001). Shear stress-induced Nitric oxide release triggers the liver regeneration cascade. *Nitric Oxide: Biology and Chemistry*. **5(5)**: 453-464.

Seyle H. Diseases of adaption. (1950). *Wisconsin medical journal*. **49(6)**: 515-516

Stockham M A. (1964).Changes of plasma and adrenal corticosterone levels in the rat after repeated stimuli. *J. Physiol.* **173**: 149-159.

Swain MS. Stress and the gastrointestinal tract. 1. (2000). Stress and hepatic inflammation. *American Journal of Physiology.* **279**: 1135-1138.

Tsigos c, Chrousos GP. (2002). Hypothalamic-Pituitary-Adrenal Axis, neuroendocrine factors, and stress. *Journal of psychosomatic Research*. **53**: 865-871.

Vincent PR, Koichi I, Hizaki K, Tetsurou N, Shin-ichi N and Gakuji I. (2002). Emotional stress and brux-like activity of the masseter muscle in rats. *European Journal of Orthodontics* . **24**: 107-117