2. Hamaguchi T, Kano M, Rikimaru H, Kanazawa M, Itoh M, Yanai K, Fukudo S: **Brain activity during distention of the descending colon in humans.** *Neurogastroenterol Motil* 2004, **16**:299-309.
3. Creed F, Guthrie E: **Psychological factors in the irritable bowel syndrome.** *Gut* 1987, **28**:1307-1318.
4. Drossman DA: **Personality and psychological factors in the irritable bowel syndrome.** *Gastroenterol Clin Biol* 1990, **14**:49C-53C.
5. Ditto B, Miller SB, Barr RG: **A one-hour active coping stressor reduces small bowel transit time in healthy young adults.** *Psychosom Med* 1998, **60**:7-10.
6. Mayer EA, Craske M, Naliboff BD: **Depression, anxiety, and the gastrointestinal system.** *J Clin Psychiatry* 2001, **62(Suppl 8)**:28-36.
7. Andresen V, Camilleri M: **Irritable bowel syndrome: recent and novel therapeutic approaches.** *Drugs* 2006, **66**:1073-1088.
8. Kano M, Fukudo S, Kanazawa M, Endo Y, Narita H, Tamura D, Hongo M: **Changes in intestinal motility, visceral sensitivity and minor mucosal inflammation after fasting therapy in a patient with irritable bowel syndrome.** *J Gastroenterol Hepatol* 2006, **21**:1078-1079.
9. Fukudo S: **Role of corticotropin-releasing hormone in irritable bowel syndrome and intestinal inflammation.** *J Gastroenterol* 2007, **42(Suppl 17)**:48-51.
10. Sagami Y, Shimada Y, Tayama J, Nomura T, Satake M, Endo Y, Shoji T, Karahashi K, Hongo M, Fukudo S: **Effect of a corticotropin releasing hormone receptor antagonist on colonic sensory and motor function in patients with irritable bowel syndrome.** *Gut* 2004, **53**:958-964.
11. Mertz H: **Psychotherapeutics and serotonin agonists and antagonists.** *J Clin Gastroenterol* 2005, **39**:S247-250.
12. Park JH, Rhee PL, Kim HS, Lee JH, Kim YH, Kim JJ, Rhee JC, Kang EH, Yu BH: **Increased beta-adrenergic sensitivity correlates with visceral hypersensitivity in patients with constipation-predominant irritable bowel syndrome.** *Dig Dis Sci* 2005, **50**:1454-1460.
13. Majdoubi ME, Metz-Boutigue MH, Garcia-Sablone P, Theodosis DT, Aunis D: **Immunocytochemical localization of chromogranin A in the normal and stimulated hypothalamo-neurohypophysial system of the rat.** *J Neurocytol* 1996, **25**:405-416.
14. Sato F, Kanno T, Nagasawa S, Yanaihara N, Ishida N, Hasegawa T, Iwanaga T: **Immunohistochemical localization of chromogranin a in the acinar cells of equine salivary glands contrasts with rodent glands.** *Cells Tissues Organs* 2002, **172**:29-36.
15. Kanamaru Y, Kikukawa A, Shimamura K: **Salivary chromogranin-A as a marker of psychological stress during a cognitive test battery in humans.** *Stress* 2006, **9**:127-131.
16. Zhang HQ, Murray GM, Coleman GT, Turman AB, Zhang SP, Rowe MJ: **Functional characteristics of the parallel SI- and SII-projecting neurons of the thalamic ventral posterior nucleus in the marmoset.** *J Neurophysiol* 2001, **85**:1805-1822.
17. Saito K, Kasai T, Nagura Y, Ito H, Kanazawa M, Fukudo S: **Corticotropin-releasing hormone receptor 1 antagonist blocks brain-gut activation induced by colonic distention in rats.** *Gastroenterology* 2005, **129**:1533-1543.
18. Watanabe S, Hattori T, Kanazawa M, Kano M, Fukudo S: **Role of histaminergic neurons in hypnotic modulation of brain processing of visceral perception.** *Neurogastroenterol Motil* 2007, **19**:831-838.
19. Watanabe S, Fukudo S: **Abnormal relationship between dissociation and hypnotic susceptibility in irritable bowel syndrome.** *Scand J Gastroenterol* 2006, **41**:757-758.
20. Carlson CR, Collins FL Jr, Nitz AJ, Sturgis ET, Rogers JL: **Muscle stretching as an alternative relaxation training procedure.** *J Behav Ther Exp Psychiatry* 1990, **21**:29-38.
21. Carlson CR, Curran SL: **Stretch-based relaxation training.** *Patient Educ Couns* 1994, **23**:5-12.
22. Ghoncheh S, Smith JC: **Progressive muscle relaxation, yoga stretching, and ABC relaxation theory.** *J Clin Psychol* 2004, **60**:131-136.
23. Whorwell PJ, Prior A, Faragher EB: **Controlled trial of hypnotherapy in the treatment of severe refractory irritable-bowel syndrome.** *Lancet* 1984, **2**:1232-1234.
24. Sugano A, Nomura T: **Influence of water exercise and land stretching on salivary cortisol concentrations and anxiety in chronic low back pain patients.** *J Physiol Anthropol Appl Human Sci* 2000, **19**:175-180.
25. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC: **Functional bowel disorders.** *Gastroenterology* 2006, **130**:1480-1491.
26. Roberts JM, Wilson K: **Effect of stretching duration on active and passive range of motion in the lower extremity.** *Br J Sports Med* 1999, **33**:259-263.
27. Sullivan MK, Dejulia JJ, Worrell TW: **Effect of pelvic position and stretching method on hamstring muscle flexibility.** *Med Sci Sports Exerc* 1992, **24**:1383-1389.
28. Svedlund J, Sjodin I, Dotevall G: **GSRS – a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease.** *Dig Dis Sci* 1988, **33**:129-134.
29. Zung WW: **A Self-Rating Depression Scale.** *Arch Gen Psychiatry* 1965, **12**:63-70.
30. Spielberger CD: *Manual for the State-Trait Anxiety Inventory (STAI)* Palo Alto, CA: Consulting Psychologists Press; 1983.
31. Talley N: **Optimal design of treatment trials.** In *The Functional Gastrointestinal Disorders: Diagnosis, Pathophysiology, and Treatment* Edited by: Drossman D, Richter J, Talley N, Thompson WEC, Whitehead W. Boston: Little, Brown and Company; 1994:265-310.
32. Machnicki G, Pefaur J, Gaite L, Linchenco AM, Raimondi C, Schiavelli R, Otero A, Margolis MK: **Gastrointestinal (GI)-Specific patient reported outcomes instruments differentiate between renal transplant patients with or without GI symptoms: results from a South American cohort.** *Health Qual Life Outcomes* 2008, **6**:53.
33. Gabrys JB, Peters K: **Reliability, discriminant and predictive validity of the Zung Self-rating Depression Scale.** *Psychol Rep* 1985, **57**:1091-1096.
34. Fountoulakis KN, Papadopoulou M, Kleanthous S, Papadopoulou A, Bizeli V, Nimatoudis I, Iacovides A, Kaprinis GS: **Reliability and psychometric properties of the Greek translation of the State-Trait Anxiety Inventory form Y: preliminary data.** *Ann Gen Psychiatry* 2006, **5**:2.
35. Guinard JX, Zoumas-Morse C, Walchak C: **Relation between parotid saliva flow and composition and the perception of gustatory and trigeminal stimuli in foods.** *Physiol Behav* 1997, **63**:109-118.
36. Yanaihara H, Hata M, Nishikawa Y, Hoshino M, Yanaihara N, Murai M: **Application of region-specific immunoassay for human chromogranin A: substantial clue for detection and measurement of chromogranin A in human plasma.** *Regul Pept* 1999, **80**:83-90.
37. Toda M, Morimoto K, Nagasawa S, Kitamura K: **Change in salivary physiological stress markers by spa bathing.** *Biomed Res* 2006, **27**:11-14.
38. Toda M, Morimoto K: **Effect of lavender aroma on salivary endocrinological stress markers.** *Arch Oral Biol* 2008, **53**:964-968.
39. Fujimoto S, Nomura M, Niki M, Motoba H, Ieishi K, Mori T, Ikefuji H, Ito S: **Evaluation of stress reactions during upper gastrointestinal endoscopy in elderly patients: assessment of mental stress using chromogranin A.** *J Med Invest* 2007, **54**:140-145.
40. Den R, Toda M, Nagasawa S, Kitamura K, Morimoto K: **Circadian rhythm of human salivary chromogranin A.** *Biomed Res* 2007, **28**:57-60.
41. Kanno T, Asada N, Yanase H, Iwanaga T, Yanaihara N: **Salivary secretion of chromogranin A. Control by autonomic nervous system.** *Adv Exp Med Biol* 2000, **482**:143-151.
42. Winkler H, Fischer-Colbrie R: **The chromogranins A and B: the first 25 years and future perspectives.** *Neuroscience* 1992, **49**:497-528.
43. Drossman DA, McKee DC, Sandler RS, Mitchell CM, Cramer EM, Lowman BC, Burger AL: **Psychosocial factors in the irritable bowel syndrome. A multivariate study of patients and nonpatients with irritable bowel syndrome.** *Gastroenterology* 1988, **95**:701-708.
44. Saito K, Kanazawa M, Fukudo S: **Colorectal distention induces hippocampal noradrenaline release in rats: an in vivo microdialysis study.** *Brain Res* 2002, **947**:146-149.
45. Elsenbruch S, Holtmann G, Oezcan D, Lysson A, Janssen O, Goebel MU, Schedlowski M: **Are there alterations of neuroendocrine and cellular immune responses to nutrients in women with irritable bowel syndrome?** *Am J Gastroenterol* 2004, **99**:703-710.

46. Lucas A, Cobelens PM, Kavelaars A, Heijnen CJ, Holtmann G, Haag S, Gerken G, Langhorst J, Dobos GJ, Schedlowski M, Elsenbruch S: **Disturbed in vitro adrenergic modulation of cytokine production in inflammatory bowel diseases in remission.** *J Neuroimmunol* 2007, **182**:195-203.
47. Talley NJ: **Serotoninergic neuroenteric modulators.** *Lancet* 2001, **358**:2061-2068.
48. Bellini M, Rappelli L, Blandizzi C, Costa F, Stasi C, Colucci R, Giannaccini G, Marazziti D, Betti L, Baroni S, *et al.*: **Platelet serotonin transporter in patients with diarrhea-predominant irritable bowel syndrome both before and after treatment with alosetron.** *Am J Gastroenterol* 2003, **98**:2705-2711.
49. Atkinson W, Lockhart S, Whorwell PJ, Keevil B, Houghton LA: **Altered 5-hydroxytryptamine signaling in patients with constipation- and diarrhea-predominant irritable bowel syndrome.** *Gastroenterology* 2006, **130**:34-43.
50. Levy RL, Linde JA, Feld KA, Crowell MD, Jeffery RW: **The association of gastrointestinal symptoms with weight, diet, and exercise in weight-loss program participants.** *Clin Gastroenterol Hepatol* 2005, **3**:992-996.
51. Kano M, Hamaguchi T, Itoh M, Yanai K, Fukudo S: **Correlation between alexithymia and hypersensitivity to visceral stimulation in human.** *Pain* 2007.
52. Engel L, Andersen LB: **Effects of body-mind training and relaxation stretching on persons with chronic toxic encephalopathy.** *Patient Educ Couns* 2000, **39**:155-161.
53. Carlson CR, Ventrella MA, Sturgis ET: **Relaxation training through muscle stretching procedures: a pilot case.** *J Behav Ther Exp Psychiatry* 1987, **18**:121-126.
54. Fukudo S: **Sex and gender in irritable bowel syndrome.** *J Gastroenterol* 2006, **41**:608-610.
55. Chang L, Toner BB, Fukudo S, Guthrie E, Locke GR, Norton NJ, Sperber AD: **Gender, age, society, culture, and the patient's perspective in the functional gastrointestinal disorders.** *Gastroenterology* 2006, **130**:1435-1446.
56. Gershon MD, Tack J: **The serotonin signaling system: from basic understanding to drug development for functional GI disorders.** *Gastroenterology* 2007, **132**:397-414.
57. Drossman DA: **The functional gastrointestinal disorders and the Rome III process.** *Gastroenterology* 2006, **130**:1377-1390.
58. Moller M, Ekstrand J, Oberg B, Gillquist J: **Duration of stretching effect on range of motion in lower extremities.** *Arch Phys Med Rehabil* 1985, **66**:171-173.
59. Bonnar BP, Deivert RG, Gould TE: **The relationship between isometric contraction durations during hold-relax stretching and improvement of hamstring flexibility.** *J Sports Med Phys Fitness* 2004, **44**:258-261.
60. Schell E, Theorell T, Hasson D, Arnetz B, Saraste H: **Stress biomarkers' associations to pain in the neck, shoulder and back in healthy media workers: 12-month prospective follow-up.** *Eur Spine J* 2008, **17**:393-405.

bat5/zpy-psy/zpy-psy/zpy00309/zpy3752-09z | xppws | S=1 | 1/23/09 | 4:49 | 4/Color Figure(s): F1-F3 | Art: PSY200532 | Input-sk

Brain Activation Covariated With Changes in Heart Rate, Heart Rate Variability, and Plasma Catecholamines During Rectal Distention

Hideaki Suzuki, MD, Satoshi Watanabe, PhD, Toyohiro Hamaguchi, PhD, Hirotaka Mine, MS, Takahiro Terui, MD, Motoyori Kanazawa, MD, PhD, Noriko Oohisa, BSN, Mitsuya Maruyama, ME, Tomoyuki Yambe, MD, PhD, Masatoshi Itoh, PhD, and Shin Fukudo, MD, PhD

Objective: To test the hypothesis that gut stimulation provokes autonomic arousal via activation of regional cerebral cortices. How the human brain processes interoceptive signals and forms initial autonomic arousal is one of the key questions to be answered in research on emotion. **Methods:** Twelve healthy males participated in this study. A barostat bag was inserted in the rectum and intermittently inflated with 0, 20, or 40 mm Hg at random for 80 seconds. $H_2{}^{15}O$ positron emission tomography (PET) of the brain, electrocardiography, and blood sampling for catecholamines were performed. Changes in regional cerebral blood flow were interpreted using statistical parametric mapping. **Results:** Rectal distention with 40 mm Hg induced a significant increase in heart rate, low-frequency (LF)/high-frequency (HF) ratio of heart rate variability, and plasma adrenaline. Activated brain areas that were covariated with increased heart rate during rectal distention were the right insula, right operculum, right dorsolateral prefrontal cortex, putamen, thalamus, periaqueductal gray, and cerebellum ($p < .001$, uncorrected), whereas those that were covariated with increased LF/HF ratio were the bilateral insula, putamen, thalamus, midbrain, pons, and cerebellum ($p < .001$, uncorrected). Activated brain areas that were covariated with increased plasma adrenaline were the right insula, right orbitofrontal cortex, right parahippocampal gyrus, putamen, thalamus, periaqueductal gray, pons, and cerebellum ($p < .001$, uncorrected). **Conclusion:** Our results suggest that the right insula and the related body mapping regions may form the functional module of sympathetic arousal in response to gut stimulation. **Key words:** positron emission tomography, heart rate, heart rate variability, catecholamine, visceral perception, rectal distention.

PET = positron emission tomography; **rCBF** = regional cerebral blood flow; **BA** = Brodmann's area; **ECG** = electrocardiogram; **HRV** = heart rate variability; **HF** = high-frequency component of HRV; **LF** = low-frequency component of HRV; **LF/HF** = ratio of LF to HF; **ANOVA** = analysis of variance.

INTRODUCTION

Emotion was proposed to have two components, one as the bodily state and the other as the feeling (1). The bodily state, which is mediated by a family of peripheral, autonomic, endocrine, and skeletomotor responses, has been believed to involve subcortical structures: the amygdala, the hypothalamus, and the brainstem whereas the feeling involves cerebral cortex.

However, neuroscience and patient studies indicated that autonomic response, the bodily state, is associated with cortical brain regions which are important in the feeling (2–5). Electrical stimulation of brain regions like insula, anterior cingulate cortex, or prefrontal cortex induced changes in blood pressure and heart rate occasionally accompanied by subjective mood changes (2,3). Patients with dysfunction of prefrontal cortex or anterior cingulate cortex did not show autonomic changes in mental tasks, which elicit autonomic arousal in normal healthy subjects (4,5). Besides, the patient with damaged prefrontal cortex could not have emotional feelings (4). These cortical brain structures are thought to play a salient role in the processing of the autonomic response as well as the feelings (4,6).

Studies using functional neuroimaging technique have examined noninvasively the relationship between autonomic arousal and brain activity. Hand gripping, mental arithmetics, mental tasks, or Valsalva maneuver provoked activation of anterior cingulate cortex, insula, prefrontal cortex, amygdala, hippocampus, cerebellum, and brainstem that were associated with autonomic activity (7–10). Stimulation of the gastrointestinal tract also provokes autonomic changes (11–13) as well as visceral sensation. Functional imaging studies have identified brain areas activated during stimulation of the esophagus (14), stomach (15), descending colon (16), or rectum (17,18). These brain areas include the anterior cingulate cortex, insula, prefrontal cortex, cerebellum, and brainstem (14–18). However, no study has ever examined the association between activation of brain regions and autonomic activity during gastrointestinal stimulation.

Recently, the processing of emotion has been conceptualized as hierarchical structures, visceral sensation, action tendencies, unidimentional and multidimentional processing, and integration of multidimentional processing (6). Emotion and identified brain regions that were covariated with autonomic changes in earlier studies are multidimensional because complex cognitive tasks were used (7–10). Therefore, identified brain regions that were covariated with autonomic changes in earlier studies should be reexamined in the lower hierarchy of emotional processing, e.g., visceral sensation.

Heart rate variability (HRV) and galvanic skin conductance have already been used as indices of autonomic activity in the previous human study of rectal distention (13). Changes in heart rate during rectal distention and the association between brain activity and serum catecholamine levels have not been studied yet in humans.

From the Department of Behavioral Medicine (H.S., S.W., T.H., H.M., T.T., M.K., S.F.); Institute of Development, Aging and Cancer (M.M., T.Y.); Tohoku University, Sendai, Japan; and Post-Graduate Education Center (H.S.) and Department of Laboratory Examination (N.O.), Tohoku University Hospital, Sendai, Japan.

Address correspondence and reprint requests to Shin Fukudo, Department of Behavioral Medicine, Tohoku University, 2-1 Seiryo, Aoba, Sendai 980-8575, Japan. E-mail: sfukudo@mail.tains.tohoku.ac.jp

Received for publication March 31, 2008; revision received September 2, 2008.

Supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture and from the Ministry of Health, Welfare, and Labor of Japan.

AQ: 2

DOI: 10.1097/PSY.0b013e31819b69ca

<zdoi;10.1097/PSY.0b013e31819b69ca> • <zjs;Original Article> • <zjss;Original Article>

ba#5/zpy-psy/zpy-psy/zpy00309/zpy3752-09z | xppws | S=1 | 1/23/09 | 4:49 | 4/Color Figure(s): F1-F3 | Art: PSY200532 | Input-sk

In this study, we tested the following hypotheses:

1. Rectal distention provokes changes in heart rate, HRV, and serum catecholamine levels in healthy young men.
2. Activated brain regions that were covariated with changes in heart rate, HRV, and serum catecholamine levels during rectal distention are the anterior cingulated cortex, insula, prefrontal cortex, amygdala, hippocampus, cerebellum, and brainstem.

MATERIALS AND METHODS

Subjects

Twelve healthy male volunteers (age = 22 ± 1 (standard error of the mean) years) were recruited to participate in this study through local advertisement between July 2004 and June 2005. Each volunteer underwent a basic medical history, medical interview, and physical examination to exclude subjects with organic diseases. All subjects were right handed and free from signs or symptoms of gastrointestinal, cardiovascular, or psychotic disorders. They were free of medication and no subject had been taking illegal drugs, smoking, drinking alcohol heavily, or taking excessive caffeine. All subjects gave their informed consent before starting the study. This study, which is a part of the Brain Imaging Project for Irritable Bowel Syndrome in Tohoku University (Principal Investigator, S.F.), was approved by the Ethics Committee of Tohoku University School of Medicine.

Experimental Design

On the day before the examination, the subjects took a low residue diet. At night (9 PM), they ingested 17 (13.6%) g of magnesium citrate, 75 mg of sodium picosulfate, and 24 mg of sennoside A & B to cleanse the colon. The subjects were then fasted overnight. The experiment began the next day at 10 AM. First, the subjects lay quietly on a bed for positron emission tomography (PET) scan at the Cyclotron Radioisotope Center, Tohoku University. Two polyethylene catheters were inserted into the bilateral cubital vein. A saline drip infusion was started at a rate of 1 ml/minute. A plastic catheter with a thin polyethylene bag (Synectics Medical, Stockholm, Sweden) was inserted into the rectum of each subject. After a radioactive tracer ($H_2{}^{15}O$) was injected through the right cubital vein, a PET scan of the brain was performed four times with or without rectal distention. Scanning time was set to 70 seconds. Approximately a 10-minute interval was given between successive distentions to ensure that radioactivity levels returned to baseline before starting a new scan. Holter electrocardiogram (ECG) was recorded throughout the experiment. Heart rate and HRV during rectal distention were analyzed later. Immediately after each distention of the rectum, blood was withdrawn via the left cubital vein for later analysis of plasma catecholamines.

Rectal Distention

Rectal distentions were induced with a computerized barostat (Medtronics Synectics, Shoreview, Minnesota), which inflated the thin polyethylene bag at rate of 38 ml s^{-1}. The maximal volume of the barostat bag was 500 ml and the maximal diameter of the bag at full inflation was 10 cm. The first stimulus was always without rectal distention (namely, baseline). Subjects then received rectal distention with an intensity of 0 (sham stimulation), 20, or 40 mm Hg. The intensities of three rectal distentions were ordered randomly to avoid order effect. Average intensities of second, third, and fourth stimulations are not significantly different among each other in one-way analysis of variance (ANOVA). There was a lag time of 6 seconds before reaching peak pressure after initiation of the stimuli. The stimuli continued for 80 seconds, a period which matched the duration of the PET scan.

Heart Rate and HRV

Data were analyzed from the recorded Holter ECG, and stimulation was marked with a specific key input. Premature ventricular or supraventricular contractions were reduced by a signal analyzer (SCM 6000, Fukuda Denshi, Tokyo). R-R intervals during stimulation were calculated by a computer software (R-R Interval Analyzing Program, HPS-RRA, Fukuda Denshi, Tokyo), which provided values for 64 seconds. Heart rate and HRV were then obtained at each four stimulation. Overall spectral analysis was applied to compute the major frequency components of HRV signal, the low-frequency (LF) band (0.04–0.15 Hz), the high-frequency (HF) band (0.15–0.4 Hz), and LF/HF ratio. The LF is under the sympathetic and parasympathetic control, whereas the HF is under the parasympathetic control (19–21). Increased LF/HF ratio reflects an increase in cardiac sympathetic tone (21,22).

Plasma Catecholamines

Blood (16 ml) was withdrawn from the left cubital vein immediately after each distention, mixed with disodium ethylenediamine tetreaacetic acid, and centrifuged at 3000 rpm at 4°C. Separated plasma was then frozen and stored at −40°C. On the day of assay, the frozen plasma was defrosted, and plasma catecholamines were determined using high performance liquid chromatography with electrochemical detection after batch alumina extraction. Detection limits of adrenaline and noradrenaline were 2.56 pg/ml and 1.35 pg/ml, respectively. Intra-assay variances of adrenaline and noradrenaline were 0.50% and 0.55%, respectively. Interassay variances of adrenaline and noradrenaline were 1.77% and 2.27%, respectively.

PET Scan

The method for brain imaging was essentially the same as that described in our previous studies (16,23). A plaster head support was set for each subject to minimize head movements during PET imaging. $H_2{}^{15}O$ (Tohoku University Cyclotron Radioisotope Center, Sendai, Japan) was injected into the right arm vein at the beginning of rectal distention. Ten seconds later, both radioactivity and peak pressure of the bag reached a plateau. As the radioactivity detected in the brain is proportional to the volume of regional cerebral blood flow (rCBF) (24), an increase in rCBF is seen as an index of neural activity evoked by stimulation (25,26). Using a $^{68}Ge/^{68}Ga$ radiation source, transmission scan for γ-ray absorption was corrected before PET scanning. PET scanning room was darkened and the subjects, while awake, were instructed to keep their eyes closed for the whole period of the scan (70 seconds). The rCBF in each subject was measured during four scans (70 seconds each), using a PET scanner in three-dimension sampling mode (HEADTOME V SET-2400W, Shimizu, Kyoto, Japan). The scanner produced 63 horizontal slices with a separation of 3.125 mm, an axial field of view of 200 mm, an in-plate resolution of 590 mm, a full width at half maximum (FWHM), and an axial resolution of 3.9 mm FWHM (27).

PET data were transferred to a super computer (NEC, SX-4/128H4, Tohoku University Computer Center, Sendai, Japan) and PET images were reconstructed, using three-dimensional filtered back projection algorithm (28–30). PET images were analyzed according to the method of Friston et al. (31–36), using a statistical parametric mapping software (SPM2, Wellcome Department of Cognitive Neurology, London, UK). PET images were realigned, spatially normalized, and transformed into approximates in Talairach-Tournoux stereotactic space (37). Finally, the images were smoothed by a 3D Gaussian filter (FWHM = 13 mm) and proportionally scaled to account for global confounders.

Analysis

Values of changes in heart rate, LF, HF, LF/HF ratio, and plasma levels of catecholamines were analyzed by one-way ANOVA. In cases where significant interactions were found in the ANOVAs, post hoc analysis using Ryan's method ($p < .05$) were conducted to examine which combinations of rectal distention intensities differed significantly. To estimate rCBF differences between baseline and each rectal distention, an intragroup comparison was made using "population main effect: two conditions, one scan/condition (paired t test)" SPM model. To evaluate the covariation between heart rate, LF/HF ratio, or catecholamine levels and rCBF during two conditions (baseline and intensity of rectal distention), regression with all ratings was performed by entering the values of heart rate, LF/HF ratio, and catecholamine levels as covariates of interest in "multi subjects, covariate only" SPM model (38). First, a level of significance was set at ≤0.1% (uncorrected for multiple comparisons) as the region of significant correction. Second, much more significant analysis were performed with a level of significance of ≤5% with

bat5/zpy-psy/zpy-psy/zpy00309/zpy3752-08z | xppws | S=1 | 1/23/09 | 4:49 | 4/Color Figure(s): F1-F3 | Art: PSY200532 | Input-sk

TABLE 1. Changes in Heart Rate, Heart Rate Variability, and Plasma Catecholamines Induced by Rectal Distention

Parameters/Bag Pressure	Baseline (0 mm Hg)	Sham (0 mm Hg)	Mild (20 mm Hg)	Intense (40 mm Hg)
Heart rate (bpm)	58.5 ± 3.8	60.3 ± 3.9	62.2 ± 3.5	72.0 ± 4.8*
LF (bpm^2)	1723 ± 801	2429 ± 889	3043 ± 1825	1293 ± 491
HF (bpm^2)	2705 ± 1259	2070 ± 846	2129 ± 816	489 ± 134
LF/HF	0.98 ± 0.30	1.68 ± 0.40	1.42 ± 0.35	2.85 ± 0.54*
adrenaline (pg/ml)	29 ± 5	27 ± 5	30 ± 7	44 ± 9*
noradrenaline (pg/ml)	196 ± 24	185 ± 21	212 ± 25	216 ± 21

Values are mean ± standard error of the mean (n = 12).
HR = heart rate; LF = low-frequency power expressed as an integrated area; HF = high-frequency power expressed as an integrated area; LF/HF = area power ratio; bpm = beats/minute.
Values significantly different from the baseline are shown as follows: *$p < .001$.

TABLE 2. Activated Brain Areas That Were Significantly and Positively Covariated With Increased Heart Rate During Rectal Distention With 40 mm Hg

Region (Brodmann Area)	Side	Coordinates (x, y, z)	T Score	Voxels in Cluster
Cerebellum*** (vermis)	—	0, −54, −22	19.50	2162
Cerebellum*	R	28, −44, −50	6.45	126
Cerebellum*	R	8, −54, −50	5.18	36
Middle insula**	R	36, 0, −2	7.95	612
Periaqueductal grey**	—	−2, −30, 0	7.87	229
Primary motor cortex** (^{4})	L	−12, −30, 70	7.78	49
Operculum* (^{42})	R	38, −14, 20	7.06	168
Dorsolateral prefrontal cortex* (^{10,46})	R	46, 58, 6	5.65	50
Putamen*	L	−20, 6, 18	5.57	79
Supplementary motor cortex* (^{8})	L	−58, 12, 38	5.08	21
Thalamus*	L	−16, −32, 16	5.24	157
Thalamus*	L	−10, −6, 6	4.57	28

Coordinates refer to location in stereotaxic space. The table shows maxima of search values. Height threshold: T = 4.02, $p < .001$. Extent threshold k = 20 voxels, $p < .234$ (uncorrected). Corrected $p < .05$*, 0.01**, and 0.001*** for multiple comparisons.

correction for multiple comparisons. Significantly activated regions were identified on the basis of Talairach coordinates (37).

RESULTS

Changes in Heart Rate, HRV, and Plasma Catecholamines Levels Induced by Rectal Distention

Rectal distention with an intensity of 40 mm Hg produced significant increases in heart rate ($p < .001$), LF/HF ratio ($p < .001$), and plasma adrenaline ($p < .001$), compared with baseline (Table 1). Changes in LF, HF, and plasma noradrenaline were not significant. The sham (0 mm Hg) or 20 mm Hg stimulation did not evoke any significant autonomic response.

Functional Module of the Brain in Proportion to Increase in Heart Rate

Intense rectal distention (40 mm Hg) significantly increased rCBF in the previously reported visceral pain circuit, i.e., left thalamus, middle portion of the right insula, right operculum, bilateral putamen, periaqueductal gray, cerebellar vermis, and bilateral cerebellum ($p < .001$, uncorrected, data not shown).

Brain regions that showed significant positive covariation between the increase in rCBF and that in heart rate during 40 mm Hg rectal distention are shown in Table 2. Activity in the middle portion of the right insula, right operculum, and right dorsolateral prefrontal cortex showed significant positive covariation with heart rate ($p < .001$, uncorrected) (Figure 1). In addition to these regions, rCBF in the left thalamus, periaqueductal gray, left primary motor cortex, left supplementary motor cortex, left putamen, cerebellar vermis, and right cerebellum were significantly covariated with heart rate. F1

Functional Module of the Brain in Proportion to Increase in LF/HF

Regions of the brain where the increase in rCBF significantly and positively were covariated with that in LF/HF ratio during rectal distention with 40 mm Hg are shown in Table 3. Activity in the posterior portion of the bilateral insula, right anterior insula, and bilateral putamen showed significant positive covariation with LF/HF ratio ($p < .001$, uncorrected) (Figure 2). Besides, significantly positive covariation between the increase in rCBF and that in LF/HF ratio was found in the right superior frontal gyrus, left thalamus, midbrain, pons, bilateral cerebellar hemisphere, and cerebellar vermis. T3 F2

Functional Module of the Brain in Proportion to Increase in Plasma Adrenaline

Significant positive covariation between the increase in rCBF and that in plasma adrenaline during 40 mm Hg rectal

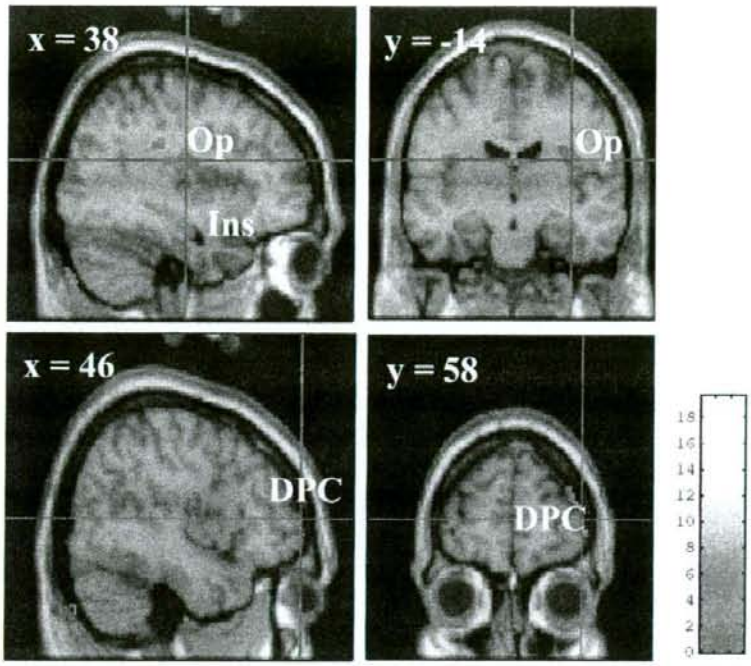


Figure 1. Activity in the middle portion of the right insula (Ins) (36, 0, −2), the right operculum (Op) (38, −14, 20), and the right dorsolateral prefrontal cortex (RPC) (46, 58, 6) positively covariated with increased heart rate during rectal distention with an intensity of 40 mm Hg. Results of covariation analysis were displayed on selected slices of the magnetic resonance imaging template available in SPM2 system. Coordinates are relative to anterior commeasure in the interaural (x), anterior-posterior (y), and superior-inferior (z) directions. Color calibration bars that apply to each image represent critical T-score magnitude of the covariated areas with a threshold voxel α level of $p < .001$ (uncorrected).

TABLE 3. Activated Brain Areas That Were Significantly and Positively Covariated With Increased LF/HF Ratio During Rectal Distention With 40 mm Hg

Region (Brodmann Area)	Side	Coordinates (x, y, z)	T Score	Voxels in Cluster
Cerebellum*	L	−18, −62, −26	9.04	180
Cerebellum*	R	32, −74, −50	8.83	434
Cerebellum (vermis)	—	10, −64, −28	5.50	346
Cerebellum	L	−14, −53, −44	5.08	53
Cerebellum	L	−38, −40, −46	4.63	27
Cerebellum	L	−46, −76, −34	4.52	42
Superior frontal gyrus* (6)	R	6, −18, 84	8.47	30
Pons*	—	−4, −32, −30	7.35	592
Putamen*	L	−20, 14, 8	7.23	310
Putamen*	R	14, 10, −8	6.42	36
Anterior insula*	R	36, −12, 4	7.07	251
Posterior insula*	L	−30, −16, 4	5.98	179
Posterior insula	R	30, 10, 12	5.03	88
Thalamus	L	−14, −32, 6	5.00	34
Midbrain region	—	16, −22, −6	4.87	52

Coordinates refer to location in stereotaxic space. The table shows maxima of search values. Height threshold: T = 4.02, $p < .001$. Extent threshold k = 20 voxels, $p < .264$ (uncorrected). Corrected $p < .05$* for multiple comparisons.

T4 distention are shown in Table 4. A significant positive covariation between the increase in rCBF and that in plasma adrenaline was detected in the anterior portion of the right insula, right orbitofrontal cortex, and right parahippocampal gyrus ($p < .001$, uncorrected) (Figure 3). Moreover, the

F3

increase in rCBF in the right superior frontal gyrus, bilateral putamen, bilateral thalamus, periagueductal gray, pons, and bilateral cerebellar hemisphere were significantly and positively covariation with the increase in plasma adrenaline.

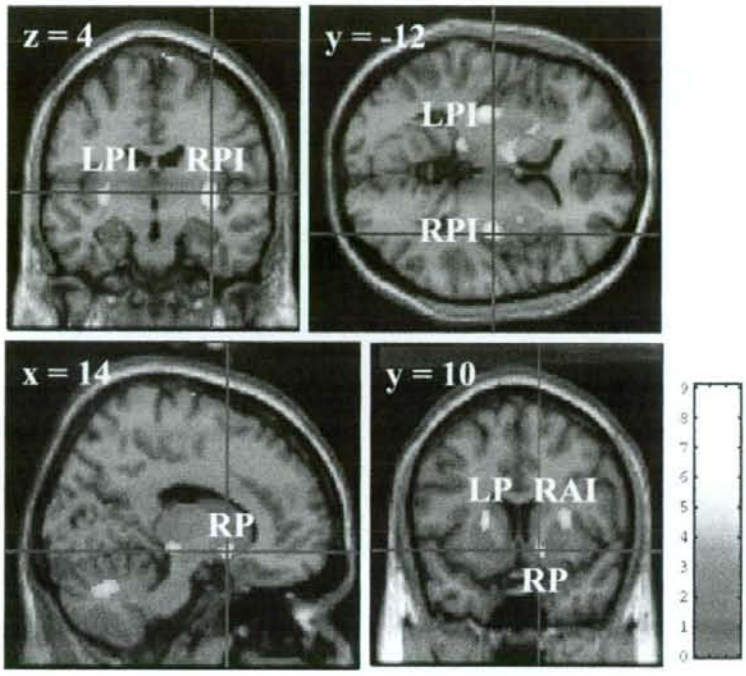


Figure 2. Activity in the right posterior insula (RPI) (36, 0, −2), the left posterior insula (LPI) (−30, −16, 4), the right anterior insula (RAI) (30, 10, 12), the left putamen (LP) (−20, 14, 8), and the right putamen (RP) (14, 10, −8) positively covariated with increased LF/HF ratio during rectal distention with an intensity of 40 mm Hg. The RP is in the caudal portion whereas the LP is more rostral. Results of covariation analysis were displayed on selected slices of the magnetic resonance imaging template available in SPM2 system. Coordinates are relative to anterior commeasure in the interaural (x), anterior-posterior (y), and superior-inferior (z) directions. Color calibration bars that apply to each image represent critical T-score magnitude of the covariated areas with a threshold voxel α level of $p < .001$ (uncorrected).

TABLE 4. Activated Brain Areas That Were Significantly and Positively Covariated With Increased Plasma Adrenaline During Rectal Distention With 40 mm Hg

Region (Brodmann Area)	Side	Coordinates (x, y, z)	T Score	Voxels in Cluster
Cerebellum*	R	14, −50, −34	8.14	1442
Cerebellum*	L	−26, −68, −50	6.13	324
Putamen*	L	−20, −2, 18	8.10	674
Putamen	R	18, −2, 10	4.36	47
Anterior insula*	R	26, 16, 18	7.24	324
Periaqueductal grey*	—	−8, −20, −10	6.30	109
Orbitofrontal cortex* (^{11})	R	28, 44, −18	6.00	25
Superior frontal gyrus* (^{6})	R	14, −12, 80	5.78	22
Thalamus*	R	16, −16, 24	5.50	332
Thalamus*	L	−18, −36, 8	4.70	52
Pons*	L	−14, −32, −28	5.43	23
Parahippocampal gyrus (^{28})	R	20, −26, −8	4.51	26

Coordinates refer to location in stereotaxic space. The table shows maxima of search values. Height threshold: T = 4.02, $p < .001$. Extent threshold k = 20 voxels, $p < .265$ (uncorrected). Corrected $p < .05$* for multiple comparisons.

DISCUSSION

This study is the first to demonstrate that cortical and subcortical brain activation was covariation with increase in three different autonomic indices, heart rate, LF/HF ratio, and plasma adrenaline during rectal distention. Regions of the brain that were significantly and positively covariated with changes in these three autonomic systems were the right insula, thalamus, putamen, periaqueductal gray, pons, and cerebellum. Regions of the brain that were covariated with heart rate only were the right operculum and the right dorsolateral prefrontal cortex, whereas those that were covariated with plasma adrenaline only were the right orbitofrontal cor-

bat5/zpy-psy/zpy-psy/zpy00306/zpy3752-08z | xppws | S=1 | 1/23/09 | 4:49 | 4/Color Figure(s): F1-F3 | Art: PSY200532 | input-sk

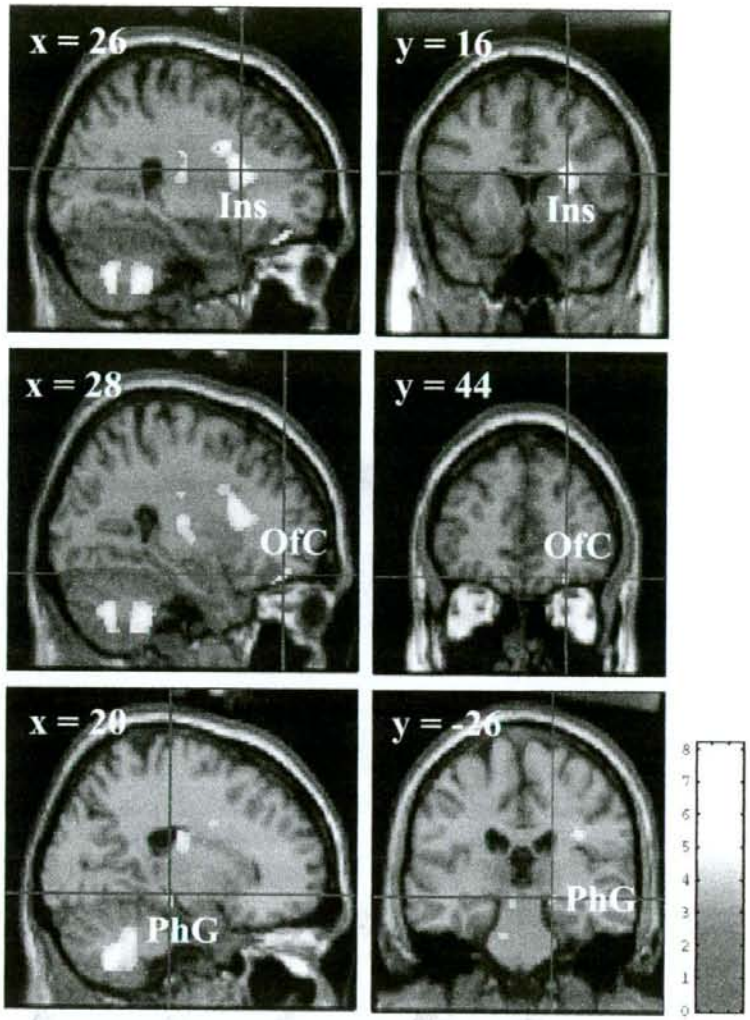


Figure 3. Activity in the anterior portion of the right insula (Ins) (26, 16, 18), the right orbitofrontal cortex (Orf) (28, 44, −18), and the right parahippocampal gyrus (PhG) (20, −26, −8) positively covariated with increased plasma adrenaline during rectal distention with an intensity of 40 mm Hg. Results of covariation analysis were displayed on selected slices of the magnetic resonance imaging template available in SPM2 system. Coordinates are relative to anterior commeasure in the interaural (x), anterior-posterior (y), and superior-inferior (z) directions. Color calibration bars that apply to each image represent critical T-score magnitude of the covariated areas with a threshold voxel α level of $p < .001$ (uncorrected).

tex and the right parahippocampal gyrus. The only region that was covariated with LF/HF ratio was the left insula. These findings show that activation of specific brain regions is associated with changes in a specific autonomic system.

Activation of the insula was covariated with increases in heart rate, LF/HF ratio, and plasma adrenaline. The insula has been reported to be involved in the processing of emotion via mapping and/or regulation of internal body states (39). In addition, the anterior portion of the insula has been shown to be involved in interoception, a sensation of body physiological conditions (40). Evidence has shown that the insula is activated during stimulation of the rectum (18) and the descending colon (16). Changes in the activity of the insula have been reported to correlate with changes in heart period during mental tasks (10). Besides, subjects' accuracy in heart beat detection task can be predicted by neural activity in the right insular/opercular cortex (41). In our previous study, activation of the insula was associated with discrimination between mild (20 mm Hg) and intense (40 mm Hg) colonic distention (16). Therefore, the insula may be activated by unusual internal signals that stimulate the sympathetic nervous system for homeostatic regulation.

rCBF COVARIATED WITH AUTONOMIC AROUSAL

Activated brain regions that were covariated with an increase in heart rate only were the right operculum and the right dorsolateral prefrontal cortex. The frontoparietal operculum is activated by esophageal stimulation and is suggested to be involved in the control of facial, masticatory, lingual, and pharyngeal musculature (13). Besides, the right opercular region is associated with interoception of heartbeats (41). The dorsolateral prefrontal cortex, on the other hand, has reciprocal connections with other brain regions including the higher-order sensory cortices (42). In our previous study, activation of the Brodmann's area (BA)10 was covariated with feelings in the gut (16). From these findings, it is suggested that the right dorsolateral prefrontal cortex and the right operculum, working in collaboration with the insula, participate in interoception-induced acceleration of heart rate. The only brain region with increased rCBF that was covariated with increased LF/HF ratio was the left insula. Increased LF/HF ratio reflects sympathetic arousal (21,22). It has been reported that sympathetic arousal is predominantly controlled by the right hemisphere (4,43). However, there are reports that indicate activity in the bilateral insula covaries with sympathetic nervous activity (9,10). LF/HF ratio is commonly believed to be associated with decrease in parasympathetic activity as well as sympathetic arousal (21). Stimulation of left insula decreased in heart rate, indicating an association with left insula and parasympathetic activity (2). Therefore, the covariation between activity in left insula and increase in LF/HF ratio in this experiment might reflect both or either decrease in parasympathetic activity of HF components or even increase in parasympathetic activity of LF components.

The only brain regions that showed increased rCBF covariated with increased plasma adrenaline were the right orbitofrontal cortex and the right parahippocampal gyrus. The orbitofrontal cortex has direct reciprocal connections with brain structures, such as the insula/operculum, the dorsolateral prefrontal cortex, the amygdala, and the hippocampus, and participates in multiple functions including the processing of emotion and sensory integration (44). The parahippocampal gyrus, on the other hand, conducts memory encoding and retrieval in cooperation with other medial temporal regions like the hippocampus and the amygdala (45). Memory encoding is strengthened by emotion, and adrenaline promotes emotional memory formation (46). Therefore, the right orbitofrontal cortex and the right parahippocampal gyrus may work together to induce arousal of emotion (gut feeling) and memory formation (unpleasant memory) accompanied by increase in plasma adrenaline during rectal distention.

In our experiments, there were activated brain regions that were covariated with increases in heart rate, LF/HF ratio, and plasma adrenaline. Among them, the thalamus, which is the gate of sensory information to the brain, is well known to be activated by visceral stimulation (16). In addition to the nucleus of solitary tract, the parabrachial nucleus in pons and the periaqueductal gray in midbrain are well-established components of the brainstem autonomic center (40,47). The periaqueductal gray regulates coordinated behavioral and autonomic responses (48), which can explain activation of motor-related brain areas accompanied by sympathetic arousal in this study. The cerebellum is also important in autonomic regulation (7). In a recent human study, patients with medial cerebellar lesions have been shown to lose fear-conditioned changes in heart rate (49). Co-occurrence of emotional flattening and autonomic reactions was also seen in a patient after left cerebellar infarction (50). Brain regions with increased rCBF that were covariated with autonomic arousal were the bilateral putamen but the right one was located more caudally than the left one. The caudal ventromedial striatum receives inputs from several limbic brain areas like the amygdala and the anterior insula, whereas the rostral striatum primarily regulates motor function (51). However, the majority of patients with pure autonomic failure and multiple system atrophy have an intact striatum (52), and electrical stimulation of the putamen does not induce remarkable changes in blood pressure or heart rate (53). Therefore, activation of the right putamen in our experiments does not directly control sympathetic regulation but may be responsible for other actions accompanied by sympathetic activity. The superior frontal gyrus (BA6) receives inputs from the insula (54), explaining the covariation of BA6 with LF/HF ratio and plasma adrenaline. Therefore, the activated brain regions except for the putamen were in plausible association with autonomic regulation and emotion during the interoception.

RCBF in the amygdala, an important component of autonomic arousal accompanied by emotion, was not covariated with changes in the three autonomic variables. There are two possible explanations for this result. The first is that activation of the amygdala might be transient in our experiments. In a fear conditioning study, firing of the amygdala was limited in the earlier phase of the experiment (55). Because PET brain image needs 70 seconds, the methodology may limit the detection. The second explanation is that the amygdala is not necessary for autonomic and emotional arousal during interoception. Although the amygdala is easily activated by fearful visual stimuli (56), its vulnerability to interoception is unknown. Most functional neuroimaging studies in gastrointestinal stimulation showed no activation of the amygdala (14–18). Therefore, the amygdala may not play as important a role in sympathetic arousal by visceral sensation as the other activated brain regions.

The important point of our study is lack of covariation between increased rCBF in the anterior cingulate cortex and changes in the three autonomic variables. The anterior cingulate cortex is known to be a motor center of the limbic system and is responsible for emotional and autonomic arousal (40). The first explanation of no detectable covariation of activity in the anterior cingulate cortex in this study is due to the fact that only male subjects participated in this study. Males show less activation of the anterior cingulate cortex in response to rectal distention than females (57). The second explanation is based on the intensity of stimulation. Vague stimulation can hardly activate the anterior cingulate cortex whereas discrete stimulation can easily fire the anterior cingulate cortex (15–17). It

was reported that activity of anterior cingulate cortex was covariated with intensity of urgency during rectal distention with 40 mm Hg in healthy male subjects (16). Thus, anterior cingulate cortex is activated to process a part of the feeling but is not associated with autonomic arousal, the bodily state, in healthy male subjects in visceral sensation, the lower hierarchy of emotional processing.

In conclusion, the results of this study support the two hypotheses we set as aims of the study: a) rectal distention provokes changes in heart rate, HRV, and serum catecholamine levels and b) brain regions that show activity, which is covariated with autonomic changes during rectal distention, are identifiable. These brain regions are: the right insula, thalamus, putamen, periaqueductal gray, pons, and cerebellum as well as the right operculum, the right dorsolateral prefrontal cortex, left insula, right orbitofrontal cortex, and right parahippocampal gyrus.

This study was presented at the 64th Annual Meeting of the American Psychosomatic Society in 2006 and was awarded the Scholar Award of the American Psychosomatic Society.

REFERENCES

1. Kandel ER, Schwartz JH, Jessell TM. Principles of neural science. In: Iversen S, Kupfermann I, Kandel ER, editors. Emotional States and Feelings. —: McGraw-Hill; 2000.
2. Oppenheimer SM, Gelb A, Girvin JP, Hachinski VC. Cardiovascular effects of human insular cortex stimulation. Neurology 1992;42:1727–32.
3. Pool JL, Ransohoff J. Autonomic effects on stimulating the rostral portion of the cingulate gyri in man. J Neurophysiol 1949;12:385–92.
4. Damasio AR. Descarts' Error: Emotion, Reason, and the Human Brain. New York: Grosset/Putnam; 1993.
5. Critchley HD, Mathias CJ, Josephs O, O'Doherty J, Zanini S, Dewar BK, Cipolotti L, Shallice T, Dolan RJ. Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. Brain 2003;126: 2139–52.
6. Lane RD. Neural correlates of conscious emotional experience. In: Lane RD, Nadel L, editors. Cognitive Neuroscience of Emotion. New York: Oxford University Press; 2000.
7. Critchley HD, Corfield DR, Chandler MP, Mathias CJ, Dolan RJ. Cerebral correlates of autonomic cardiovascular arousal: a functional neuroimaging investigation in humans. J Physiol 2000;523:259–70.
8. Critchley HD, Elliot R, Mathias CJ, Dolan RJ. Neural activity relating to generation and representation of galvanic skin conductance responses: a functional magnetic resonance imaging study. J Neurosci 2000;20: 3033–40.
9. Henderson LA, Macey PM, Macey KE, Frysinger RC, Woo MA, Harper RK, Alger JR, Yan-go FL, Harper RM. Brain responses associated with the Valsalva maneuver revealed by functional magnetic resonance imaging. J Neurophysiol 2002;88:3477–86.
10. Gianoros PJ, Van der Veen FM, Jennings JR. Regional cerebral blood flow correlates with heart period and high-frequency heart period variability during working-memory tasks: implications for the cortical and subcortical regulation of cardiac autonomic activity. Psychophysiology 2004;41:521–30.
11. Ness TJ, Gebhart GF. Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudaffective reflexes in the rat. Brain Res 1988;450:153–69.
12. Ness TJ. Intravenous lidocaine inhibits visceral nociceptive reflexes and spinal neurons in the rat. Anesthesiology 2000;92:1685–91.
13. Tillisch K, Mayer EA, Labus JS, Stains J, Chang L, Naliboff BD. Sex specific alterations in autonomic function among patients with irritable bowel syndrome. Gut 2005;54:1396–401.
14. Aziz Q, Andersson JLR, Valind S, Sundin A, Hamdy S, Jones AK, Foster ER, Langstrom B, Thompson DG. Identification of human brain loci processing esophageal sensation using positron emission tomography. Gastroenterology 1997;113:50–9.
15. Ladabaum URI, Minoshima S, Hasler WL, Cross D, Chey WD, Owyang C. Gastric distention correlates with activation of multiple cortical and subcortical regions. Gastroenterology 2001;120:369–76.
16. Hamaguchi T, Kano M, Rikimaru H, Kanazawa M, Itoh M, Yanai K, Fukudo S. Brain activity during distention of the descending colon in humans. Neurogastroenterol Motil 2004;16:299–309.
17. Silverman DHS, Munakata JA, Ennes H, Mandelkern MA, Hoh CK, Mayer EA. Regional cerebral activity in normal and pathological perception of visceral pain. Gastroenterology 1997;112:64–72.
18. Mertz H, Morgan V, Tanner G, Pickens D, Price R, Shyr Y, Kessler R. Regional cerebral activation in irritable bowel syndrome and control subjects with painful and nonpainful rectal distention. Gastroenterology 2000;118:842–8.
19. Akselrod S, Gordon D, Ubel FA, Shannon DC, Barger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science 1981;213:220–2.
20. Kamath MV, Fallen EL. Power spectral analysis of heart rate variability: a noninvasive signature of cardiac autonomic function. Crit Rev Biomed Eng 1993;21:245–311.
21. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Circulation 1996;93:1043–65.
22. Malliani A, Lombardi F, Pagani M. Power spectrum analysis of heart rate variability: a tool to explore neural regulatory mechanisms. Br Heart J 1994;71:1–2.
23. Kano M, Fukudo S, Gyoba J, Kamachi M, Tagawa M, Mochizuki H, Itoh M, Hongo M, Yanai K. Specific brain processing of facial expressions in people with alexithymia: an H2 15O-PET study. Brain 2003;126: 1474–84.
24. Collins RC. Basic aspects of functional brain metabolism. Ciba Found Symp 1991;163:6–22.
25. Fox PT, Mintun MA, Raichle ME, Miezin FM, Allman JM, Van Essen DC. Mapping human visual cortex with positron emission tomography. Nature 1986;6323:806–9.
26. Fox PT, Mintun MA, Reiman EM, Raichle ME. Enhanced detection of focal brain responses using inter subject averaging and change distribution analysis of subtracted PET images. J Cereb Blood Flow Metab 1988;8:642–53.
27. Fujiwara T, Watanuki S, Yamamoto S et al. Performance evaluation of a large axial field-of-view PET scanner: SET-2400W. Ann Nucl Med 1997;11:307–13.
28. Colsher JG. Fully three-dimensional positron emission tomography. Phys Med Biol 1980;25:103–15.
29. Kinhan PE, Rogers JG. Analytic 3D image reconstruction using all detected events. IEEE Trans Nucl Sci 1989;36:964–8.
30. Cherry SR, Dahlbom M, Hoffman EJ. Evaluation of a 3D reconstruction algorithm for multi-slice PET scanners. Phys Med Biol 1992;37:779–90.
31. Friston KJ, Frith CD, Liddle PF, Dolan RJ, Lammertsma AA, Frackowiak RSJ. The relationship between global and local changes in PET scans. J Cereb Blood Flow Metab 1990;10:458–66.
32. Friston KJ, Frith CD, Liddle PF, Frackowiak RSJ. Comparing functional (PET) images: the assessment of significant change. J Cereb Blood Flow Metab 1991;11:690–9.
33. Friston KJ, Frith CD, Liddle PF, Frackowiak RSJ. Functional connectivity: the principal-component analysis of large (PET) data sets. J Cereb Blood Flow Metab 1993;13:5–14.
34. Friston KJ, Worsley KJ, Frackowiak RSJ, Mazziotta JC, Evans AC. Assessing the significance of focal activations using their spatial extent. Hum Brain Mapp 1994;1:210–20.
35. Friston KJ, Holmes A, Worsley KJ, Poline JP, Frith CD, Frackowiak RSJ. Spatial parametric maps in functional imaging: general linear approach. Hum Brain Mapp 1995;1:189–210.
36. Friston KJ, Ashbuner J, Poline JB, Frith CD, Frackowiak RSJ. Spatial registration and normalization of images. Hum Brain Mapp 1995;2: 165–89.
37. Talairach J, Tournoux P. Co-Planar Stereotaxic Atlas of the Human Brain. New York: Thieme Medical Publishers; 1988.
38. Friston KJ, Poline JB, Holmes AP, Frith CD, Frackowiak RSJ. A multivariate analysis of PET activation studies. Hum Brain Mapp 1996;4: 140–51.
39. Damasio AR, Grabowski TJ, Bechara A, Damasio H, Ponto LL, Parvizi J, Hichwa RD. Subcortical and cortical brain activity during the feeling of self-generated emotions. Nat Neurosci 2000;3:1049–56.

halt5/zpy-psy/zpy-psy/zpy00308/zpy3752-09z | xppws | S=1 | 1/23/09 | 4:49 | 4/Color Figure(s): F1-F3 | Art: PSY200532 | Input-sk

40. Craig AD. How do you feel? Interoception: the sense of the physiological condition of the body. Nat Neurosci 2002;3:655–66.
41. Critchley HD, Wiens S, Rotshtein P, Ohman A, Dolan RJ. Neural systems supporting interoceptive awareness. Nat Neurosci 2004;7: 189–95.
42. Wood JN, Grafman J. Human prefrontal cortex: processing and representational perspectives. Nat Rev Neurosci 2003;4:139–47.
43. Hilz MJ, Dutsch M, Perrine K, Nelson PK, Rauhut U, Devinsky O. Hemispheric influence on autonomic modulation and baroreflex sensitivity. Ann Neurol 2001;49:575–84.
44. Kringelbach ML. The human orbitofrontal cortex: linking reward to hedonic experience. Nat Rev Neurosci 2005;6:691–702.
45. Larry RS, Craig ELS, Robert EC. The medial temporal lobe. Annu Rev Neurosci 2004;27:279–306.
46. Cahill L, Prins B, Weber M, Mcgaugh JL. Beta-adrenergic activation and memory for emotional events. Nature 1994;371:702–4.
47. Craig AD. Interoception: the sense of the physiological condition of the body. Curr Opin Neurobiol 2003;13:500–5.
48. Bandler R, Carrive P, Zhang SP. Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: viscerotopic, somatotopic and functional organization. Prog Brain Res 1991;87:269–306.
49. Maschke M, Schugens M, Kindsvater K, Drepper J, Kolb FP, Dienerb H-C, Daum I, Timmann D. Fear conditioned changes of heart rate in patients with medial cerebellar lesions. J Neurol Neurosurg Psychiatry 2002;72:116–8.
50. Annoni JM, Ptak R, Caladara-Schnetzer AS, Khateb A, Pollermann BZ. Decoupling of autonomic and cognitive emotional reactions after cerebellar stroke. Ann Neurol 2003;53:654–8.
51. Fudge JL, Breitbart MA, Danish M, Pannoni V. Insular and gustatory inputs to the caudal ventral striatum in primates. J Comp Neurol 2005; 490:101–18.
52. Brooks DJ, Salmon EP, Mathias CJ, Quinn N, Leenders KL, Bannister R, Marsden CD, Frackowiak RS. The relationship between locomotor disability, autonomic dysfunction, and the integrity of the striatal dopaminergic system in patients with multiple system atrophy, pure autonomic failure, and Parkinson's disease, studied with PET. Brain 1990;113: 1539–52.
53. Angyan L. Somatomotor and cardiorespiratory responses to basal ganglia stimulation in cats. Physiol Behav 1994;56:167–73.
54. Augustine JR. Circuitry and functional aspects of the insular lobe in primates including humans. Brain Res Rev 1996;22:229–44.
55. Buchel C, Morris J, Dolan R, Friston K. Brain systems mediating aversive conditioning: an event related fMRI study. Neuron 1998;20:947–57.
56. Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ, Dolan RJ. A differential neural response in the human amygdala to fearful and happy facial expressions. Nature 1996;383:812–5.
57. Kern M, Jaradeh S, Arndorfer RC, Jesmanowicz A, Hyde J, Shaker R. Gender differences in cortical representation of rectal distension in healthy humans. Am J Physiol Gastrointest Liver Physiol 2001;281: G1512–G1523.

Inflamm. res. 57, *Supplement 1* (2008) S39–S40
1023-3830/08/01S39-02
DOI 10.1007/s00011-007-0620-4

Inflammation Research

The roles of histamine H_1 receptors on cognition

K. Yanai, H. Dai, E. Sakurai and T. Watanabe

Department of Pharmacology, Tohoku University School of Medicine, Sendai 980-8575, Japan, Fax:++81 227178060, e-mail: yanai@mail.tains.tohoku.ac.jp

Published Online First 14 March 2008

Introduction

Histamine neurons are located exclusively in the posterior hypothalamus, from where they project diffusely to all regions of brain. Neuronal histamine has been implicated in a variety of brain functions including wakefulness, learning and memory [1]. Although it is well known that sedative antihistamines induce cognitive decline in humans through blockage of H_1 receptor [2], both facilitatory and inhibitory effects of neuronal histamine on learning and memory have been described in animal behavioral studies [3, 4]. Histaminergic neurotransmission has been also implicated in pathophysiology of stress-related psychiatric diseases [5]. Although several atypical antipsychotics are potent H_1 antagonists [6], the clinical significance of interaction between atypical antipsychotics and H_1 receptors is still unknown. The aim of this study was to investigate the role of histamine H_1 receptors on cognition in normal conditions using H_1 receptor gene knockout mice (H_1KO). We also investigated the effects of H_1 receptor blockade on social isolation-induced behavioral and neurochemical changes in H_1KO mice and their wild-type (WT) mice.

Materials and methods

Experiment 1: Under socially-reared normal conditions, learning and memory were evaluated in H_1KO mice by object recognition, Barnes maze and fear conditioning tests. These behavioral tasks are dependent on the function of prefrontal cortex, hippocampus or amygdala. Furthermore, we also examined long-term potentiation (LTP) in CA1 area of hippocampus in H_1KO mice and their WT mice.

Experiment 2: H_1KO and WT mice were subjected to social isolation immediately after weaning. After 4-week social isolation, locomotion, pre-pulse inhibition (PPI) of startle response and Morris water maze were evaluated. After the experiments, contents of monoamines were measured by HPLC.

All experimental protocols were approved by the Animal Care Committee of Tohoku University, and all experiments were performed in compliance with relevant laws and institutional guidelines.

Results and discussions

Results are summarized in Table 1 and Figure 1. In normal socially-reared conditions, object recognition and Barnes maze performance were significantly impaired in H_1KO mice when compared to the wild-type (WT) mice. Conversely, H_1KO mice showed better auditory and contextual freezing acquisition than their respective WT mice. LTP in CA1 area of hippocampus was significantly reduced in H_1KO mice when compared with their respective WT mice. Auditory fear conditioning tests are appropriate behavioral tasks for assessing emotional memory. Auditory fear conditioning depends on activation of the amygdala. The prefrontal cortex, where densities of H_1 receptors are high, has the potential to regulate affective processes by inhibition of lateral nucleus of amygdala. Therefore potentiation of conditioned freezing behavior was described in rats subjected to lesions of medial prefrontal cortex [7]. This work suggests that histaminergic neuron system exerts a negative influence on freezing behavior through H_1 receptors. Our results of Experiment 1 demonstrate that H_1 receptors are involved in learning and

Table 1. Effects of H_1 receptor deficiency on cognition in socially-reared normal conditions.

	Object recognition index	Latency of Barnes maze (4th Day)	Freezing time in fear conditioning	LTP
H_1KO	25 % decrease *	122 % increase *	48 % increase *	30 % decrease *

Data of H_1KO mice were expressed by the % change when compared to those in WT mice. * Significant changes

Correspondence to: K. Yanai

Table 2. Effect of social isolation on cognition in H_1KO and their WT mice.

	Locomotion	PPI (71 bB prepulse)	Latency of Morris water maze (4th Day)	Dopamine turnover rate
H_1KO mice	9 % decrease	3 % decrease	15 % increase	21 % decrease
WT mice	31 % decrease *	55 % decrease *	59 % increase *	47 % increase *

Data were expressed by the % change by social isolation when compared to group housing. * Significant changes

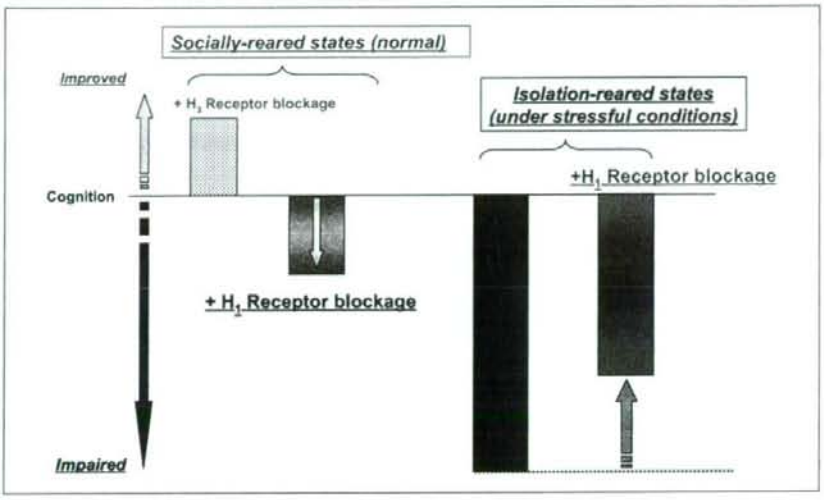


Fig. 1. Effects of histamine H_1 receptor blockage on cognition are states-dependent: A hypothesis.

memory processes for which the frontal cortex, amygdala and hippocampus interact.

In Experiment 2, locomotor activity in home cages was significantly lower in isolation-reared WT mice than in socially reared WT mice. However, no significant change in locomotor activity was observed between socially and isolation-reared H_1KO mice. Social isolation significantly impaired PPI of startle response in WT mice but not in H_1KO mice. Additionally, social isolation significantly impaired spatial learning and memory in WT mice but not in H_1KO mice. A neurochemical study revealed that isolation-reared WT mice had significantly lower dopamine (DA) levels and slightly increased DA turnover in cortex than socially reared WT mice. Conversely, isolation-reared H_1KO mice showed significantly higher DA contents as compared with socially reared H_1KO mice. Experiment 2 results indicate that blockage of H_1 receptor-mediated neurotransmission attenuates social isolation-induced behavioral and neurochemical changes and that therapeutic effects of atypical antipsychotics are mediated, at least in part, by interaction with H_1 receptors in the brain.

The conceptual hypothesis from this study is that the effects of histamine H_1 receptor blockage could be states-dependent (Fig. 1). In socially-reared normal states, blocking H_1 receptors impairs cognition. Sedative antihistamines induce cognitive decline in humans, and H_3 receptor blocking can stimulate activity of histamine neurons, and also improve cognition [8]. However, in isolation-reared states, this is under stressful condition, blockage of H_1 receptors attenuates impaired cognition induced by social isolation. This explains why both facilitatory and inhibitory effects of neuronal histamine on learning and memory have been described in animal behavioral studies.

References

[1] Haas HL, Panula P. The role of histamine and the tuberomamillary nucleus in the nervous system. Nature Rev Neurosci 2003; 4: 121–30.

[2] Yanai K, Tashiro M. The physiological and pathophysiological roles of neuronal histamine: An insight from human positron emission tomography. Pharmacol Therapeut 2007; 113: 1–15.

[3] Huston JP, Wagner U, Hasenohrl RU. The tuberomammillary nucleus projections in the control of learning, memory and reinforcement processes: evidence for an inhibitory role. Behav Brain Res 1997; 83: 97–105.

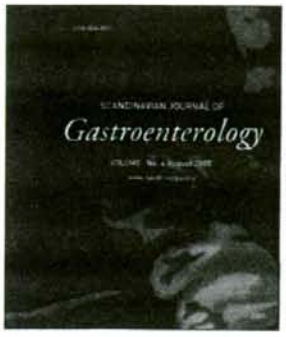
[4] Raber J. Histamine receptor-mediated signaling during development and brain function in adulthood. Cell Mol Life Sci. 2007; 64: 735–41.

[5] Prell GD, Green JP, Kaufmann CA, Khandelwal JK, Morrishow AM, Kirch DG et al. Histamine metabolites in cerebrospinal fluid of patients with chronic schizophrenia: their relationships to levels of other aminergic transmitters and ratings of symptoms. Schizophr Res 1995; 14: 93–104.

[6] Richelson E, Souder T. Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. Life Sci 2000; 68: 29–39.

[7] Vouimba RM, Garcia R, Baudry M, Thompson RF. Potentiation of conditioned freezing following dorsomedial prefrontal cortex lesions does not interfere with fear reduction in mice. Behav Neurosci 2000; 114: 720–4.

[8] Passani MB, Lin JS, Hancock A, Crochet S, Blandina P. The histamine H_3 receptor as a novel therapeutic target for cognitive and sleep disorders. Trends Pharmacol Sci 2004; 25: 618–25.



Scandinavian Journal of Gastroenterology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713690387

A randomized, double-blind, placebo-controlled clinical trial of the effectiveness of the novel serotonin type 3 receptor antagonist ramosetron in both male and female Japanese patients with diarrhea-predominant irritable bowel syndrome

Kei Matsueda [a]; Shigeru Harasawa [b]; Michio Hongo [c]; Nobuo Hiwatashi [d]; Daisuke Sasaki [e]
[a] International Medical Center of Japan Kohnodai Hospital, Ichikawa, Japan [b] Saitama-ken Saiseikai Kawaguchi General Hospital, Kawaguchi, Japan [c] Tohoku University Hospital, Sendai, Japan [d] Iwaki Kyoritsu General Hospital, Iwaki, Japan [e] Student Health & Counseling Center, Hirosaki University, Aomori, Japan

First Published:2008

To cite this Article Matsueda, Kei, Harasawa, Shigeru, Hongo, Michio, Hiwatashi, Nobuo and Sasaki, Daisuke(2008)'A randomized, double-blind, placebo-controlled clinical trial of the effectiveness of the novel serotonin type 3 receptor antagonist ramosetron in both male and female Japanese patients with diarrhea-predominant irritable bowel syndrome',Scandinavian Journal of Gastroenterology,43:10,1202 — 1211
To link to this Article: DOI: 10.1080/00365520802240255
URL: http://dx.doi.org/10.1080/00365520802240255

Scandinavian Journal of Gastroenterology, 2008; 43: 1202–1211

informa
healthcare

ORIGINAL ARTICLE

A randomized, double-blind, placebo-controlled clinical trial of the effectiveness of the novel serotonin type 3 receptor antagonist ramosetron in both male and female Japanese patients with diarrhea-predominant irritable bowel syndrome

KEI MATSUEDA[1], SHIGERU HARASAWA[2], MICHIO HONGO[3], NOBUO HIWATASHI[4] & DAISUKE SASAKI[5]

[1] *International Medical Center of Japan Kohnodai Hospital, Ichikawa, Japan,* [2] *Saitama-ken Saiseikai Kawaguchi General Hospital, Kawaguchi, Japan,* [3] *Tohoku University Hospital, Sendai, Japan,* [4] *Iwaki Kyoritsu General Hospital, Iwaki, Japan, and* [5] *Student Health & Counseling Center, Hirosaki University, Aomori, Japan*

Abstract

Objective. Irritable bowel syndrome is characterized by abdominal discomfort and/or pain associated with altered bowel habits. The neurotransmitter serotonin and serotonin type 3 receptors that are extensively distributed on enteric neurons in the human gastrointestinal tract play a role in increasing the sensation of pain and affecting bowel habits in patients with irritable bowel syndrome. The aim of this study was to evaluate the efficacy and safety of the serotonin type 3 receptor antagonist ramosetron hydrochloride in Japanese patients with diarrhea-predominant irritable bowel syndrome. ***Material and methods.*** In a double-blind, placebo-controlled, parallel group-comparative study with a 1-week run-in period, 539 patients with diarrhea-predominant irritable bowel syndrome meeting the Rome II diagnostic criteria received either 5 μg ramosetron hydrochloride ($n=270$) or placebo ($n=269$) once daily for 12 weeks. ***Results.*** Forty-seven percent of ramosetron hydrochloride-treated patients were monthly responders in the primary end-point, "Patient-reported global assessment of relief of irritable bowel syndrome symptoms", compared with 27% for placebos ($p<0.001$). The most frequently reported adverse event in the ramosetron hydrochloride-treated group compared with the placebo group was hard stool. ***Conclusions.*** Ramosetron hydrochloride 5 μg once daily is effective and well tolerated in the treatment of abdominal pain, discomfort and bowel habits in patients with diarrhea-predominant irritable bowel syndrome.

Key Words: *5-HT_3 receptor antagonist, irritable bowel syndrome, ramosetron, randomized controlled study*

Introduction

Irritable bowel syndrome (IBS) is a functional disease with persisting gastrointestinal symptoms, mainly abdominal discomfort and/or pain with abnormal bowel habits, not accompanied by organic diseases. IBS is an extremely common functional bowel disorder with an estimated prevalence of approximately 10–15% in the general population, and the annual incidence is probably 1–2% [1]. Kumano et al. reported that they found a 6.1% prevalence of IBS in total, with 7.8% in females and 4.5% in males, in a representative Japanese sample; these figures are similar to those reported in Western industrialized countries, when diagnosed using the Rome II criteria [2].

Although IBS is not a fatal disease, patients with IBS are forced to limit their behavior depending upon the extent of the symptoms, their social activity is restricted, and the health-related quality of life of IBS patients has been reported to be impaired to a level comparable with that of patients who have dialysis-dependent end-stage renal disease [3].

The mechanisms underlying the pathophysiology of IBS have not yet been fully elucidated [4]. Various psychogenic stresses have been considered to be associated with the occurrence of IBS and its symptoms. Such stress is considered to cause excitement of

Correspondence: Kei Matsueda, MD, International Medical Center of Japan Kohnodai Hospital, 1-7-1 Kohnodai, Ichikawa, Chiba 272-8516, Japan. Tel: +81 47-3723 501. Fax: +81 47 3754 775. E-mail: matsueda@imcjk.hosp.go.jp

(Received 3 April 2008; accepted 23 May 2008)

ISSN 0036-5521 print/ISSN 1502-7708 online
DOI: 10.1080/00365520802240255

the descending nerve via a release of corticotropin-releasing hormone (CRH) and to cause abnormality of motility of the digestive tract and a lowering of the threshold of perception of the digestive tract via a transmitter such as serotonin (5-HT) [5,6]. In recent years, there has been increasing interest in the possible involvement of 5-HT in this syndrome. Serotonin type 3 (5-HT_3) receptors have been identified on the sensory neurons of the gut, and they mediate reflexes that control gastrointestinal motility and secretion, bowel function and the perception of pain [7]. In patients with IBS, 5-HT_3 receptor antagonists increase colonic compliance, slow colonic transit, improve stool consistency and are valuable therapeutic compounds for treatment [8,9].

Clinical studies with the selective 5-HT_3 receptor antagonist alosetron hydrochloride (alosetron) have shown that women with diarrhea-predominant IBS (D-IBS) taking 1 mg alosetron twice daily had significantly greater adequate relief of IBS pain and discomfort and significant improvement in bowel urgency, stool form and stool frequency compared to subjects taking a placebo [10–12]. Alosetron also provided moderate-to-substantial IBS global symptom improvement in a significantly greater proportion of women with D-IBS than the placebo [13]. A comparison study with the smooth muscle relaxant mebeverine hydrochloride showed that alosetron provided greater relief of pain and of some IBS bowel abnormalities [14]. However, alosetron is indicated only for women with severe, chronic D-IBS in whom conventional therapy has failed since serious gastrointestinal events, some fatal, including ischemic colitis and bowel motor dysfunction, have been reported with alosetron use.

Ramosetron hydrochloride (Ramosetron), a tetrahydrobenzimidazole derivative, is a potent and selective 5-HT_3 receptor antagonist, and since 1996 has already been on the market in Japan and some other Asian countries as an antiemetic for cancer patients in their chemotherapy [15,16]. In preclinical studies with rats, ramosetron dose-dependently suppressed defecation disturbance induced by the stress from restraint [17]. Ramosetron also dose-dependently suppressed accelerated defecation induced by CRH, which is believed to be associated with stress-related gastrointestinal dysfunction [18]. Moreover, ramosetron significantly increased the threshold of colonic pain induced by colonic distension [19]. Recently, the efficacy and safety of ramosetron in the treatment of patients with D-IBS meeting the Rome II diagnostic criteria has been evaluated in a multicenter, double-blind, dose-ranging study in Japan comparing three doses of ramosetron (1 μg, 5 μg and 10 μg once daily, orally) with a placebo [20]. It has been shown that both 5 μg and 10 μg ramosetron were effective doses for improving overall IBS symptoms, abdominal pain, discomfort and abnormal bowel habits. Neither ischemic colitis nor severe constipation, both of which were reported with alosetron use, was observed in treatment with ramosetron.

The present, double-blind, placebo-controlled, parallel group-comparative, phase III study was conducted to verify the superiority of ramosetron, 5 μg once daily, to a placebo and to evaluate its safety in Japanese patients with D-IBS.

Material and methods

Patients

The study was conducted from August 2004 to July 2005 at multiple centers in Japan. The study protocol was designed in accordance with the Declaration of Helsinki and approved by institutional review boards at all sites. Written, informed consent was obtained from all patients before they entered the study.

Patients were eligible if aged 20–64 years and diagnosed with D-IBS (at least 3 months of D-IBS symptoms as defined by the Rome II criteria). Organic diseases were ruled out by a sigmoidoscopic examination or barium enema in patients under 49 years of age and by colonoscopic examination or barium enema in patients over 50 years of age, performed after the onset of symptoms and within the last 5 years.

Patients were excluded if they had a history of surgical resection of the stomach, or intestine (excluding appendicitis or resection of benign polyps), if they had a history of or complication from inflammatory bowel disease, ischemic colitis or malignant tumors, if they had complications from infectious enteritis, hyperthyroidism or hypothyroidism, if they used drugs that could potentially affect the evaluation of efficacy of the study drug (patients who could have a washout period of ≥ 3 days before the start of the run-in period were acceptable), if they had a history of drug or alcohol abuse within the past year or were currently abusing them, if they were allergic to drugs, had severe depression or an anxiety disorder, if they had complications from a serious cardiovascular disease, respiratory disease, renal disease, hepatic disease, gastrointestinal disease (excluding IBS), hematological disease or neurological or psychiatric disease, or if investigational products had been administered within 6 months prior to the start of this study. Female patients who were pregnant, or who were lactating were also excluded.

Study design

During the 1-week run-in period, data on abnormal bowel habits and abdominal discomfort and/or pain were collected to ensure that patients had suitable symptom levels at the start of the study. Severity of abdominal discomfort and/or pain was assessed daily on a 5-point scale (0: None, 1: Mild, 2: Moderate, 3: Severe, 4: Intolerable). Average daily scores of abdominal discomfort and/or pain during the run-in period were required to be ≥ 0.7 for patients to enter the treatment phase. Stool form (appearance) and stool frequency data were also monitored daily. Stool form data were scored on a 7-point ordinal scale according to the Bristol Stool Form Scale. Absence of stool was assigned a value of 0. Patients whose stool form was not type 1 or 2 and whose stool frequency was ≥ 3 times/week during the run-in period were enrolled, to exclude patients with predominant constipation.

Following the 1-week run-in period, eligible patients were randomly assigned 12 weeks of oral treatment with either placebo or ramosetron 5 μg tablets once daily taken before breakfast. Outpatient visits were scheduled at 2, 4, 8, and 12 weeks (or final treatment) to assess drug compliance and occurrence of adverse events.

Data collection

During the run-in period and treatment phase, patients recorded daily their IBS symptoms (severity of abdominal discomfort and/or pain, stool form, stool frequency, bowel urgency and feeling of incomplete bowel movement) on paper diary cards. Once every 7 days during the treatment phase, patients also provided weekly assessments of relief of overall IBS symptoms, abdominal discomfort and/or pain and abnormal bowel habits compared to the way they felt during the run-in period. At the same time, data input by telephone was employed to remind patients to enter data on a daily basis, or to ascertain whether the patient was experiencing a problem.

The primary end-point in the study was the monthly responder rate of "Patient-reported global assessment of relief of IBS symptoms". A monthly responder was defined as a patient who had experienced "Completely relieved" or "Considerably relieved" for at least 2 weeks of the 4-week treatment (0: Completely relieved, 1: Considerably relieved, 2: Somewhat relieved, 3: Unchanged, 4: Worsened). The primary efficacy end-point was defined by the responder rate of the last 4 weeks of the treatment phase.

Secondary end-points included the "Patient-reported assessment of relief of abdominal discomfort and/or pain", the "Patient-reported assessment of improvement of abnormal bowel habits", and assessment of IBS symptoms (severity of abdominal discomfort and/or pain, stool form, stool frequency, bowel urgency and feeling of incomplete bowel movement).

A monthly responder for the "Patient-reported assessment of relief of abdominal discomfort and/or pain" was defined as above equally as "Patient-reported global assessment of relief of IBS symptoms". For "Patient-reported assessment of improvement of abnormal bowel habits", a monthly responder was defined as a patient who was "Nearly normalized" or "Considerably relieved" for at least 2 weeks of the 4-week treatment (0: Nearly normalized, 1: Considerably relieved, 2: Somewhat relieved, 3: Unchanged, 4: Worsened).

Statistical analysis

A sample size was calculated that would provide 90% power, with a two-sided level of significance of 0.05, to detect a difference of 15% in the primary end-point between the two groups of 27% for the placebo group and 42% for the ramosetron group. A total of 460 patients (230 patients per group) or more was planned to be randomized in the study, assuming a dropout rate of a few percent.

Efficacy analyses were conducted on the full analysis set (FAS). The FAS would consist of all randomized patients who received at least one dose of study medication and had at least one post-baseline efficacy measurement.

The primary end-point was the responder rate of "Patient-reported global assessment of relief of IBS symptoms", which was compared between treatment groups by means of the χ^2 test with a two-sided level of significance of 0.05. For secondary analysis, the primary end-point was analyzed, using the χ^2 test with gender as the stratification factor.

The other monthly responder rate parameters were analyzed similarly to the primary end-point. The weekly responder variables and the continuous variables (e.g. change in average daily scores from the baseline) were summarized at each week of treatment. The average daily scores were calculated for each subsequent week of study medication. If more than two daily scores were missing during any week of study medication, the average score for that week was also defined as missing.

Results

Study population and demographics

Five hundred thirty-nine of 676 subjects who provided written, informed consent to participate

in the study were enrolled in the treatment phase. A flowchart of patient progression through the study is presented in Figure 1. Of those who dropped out of the study before or during the run-in period, 48 did not meet the inclusion criteria, 3 withdrew consent, 1 had adverse events and 85 were excluded for other reasons.

A total of 229 (85%) of 270 and 223 (83%) of 269 of 539 randomized patients in the ramosetron and placebo groups, respectively, completed the study. Forty-one patients prematurely discontinued treatment in the ramosetron group and 46 in the placebo-treated group. Reasons for premature discontinuation from the study are indicated in Figure 1.

The demographic and baseline characteristics data for randomized patients were comparable for both treatments (Table I). Patients were predominantly male and in their 30–40s. Male patients seemed to have had IBS for a longer time than female patients.

Efficacy

Primary efficacy evaluation: patient-reported global assessment of relief of IBS symptoms. Forty-seven percent of ramosetron-treated patients were monthly responders at the final point (last 4 weeks) compared with 27% for placebo (treatment difference of 20 percentage points; $p<0.001$; Figure 2). The monthly responder rates at each month were also significantly higher in the ramosetron group than in the placebo group.

We further assessed weekly response rates to evaluate onset and sustainability of response (Figure 3). Patients assessed IBS symptoms every 7 days. According to their global assessment scores, patients who had complete or considerable relief were defined as weekly responders in that particular week. Ramosetron provided greater weekly response rates than placebo. Improvement was achieved by the first week of treatment and was sustained throughout the 12 weeks of treatment.

Figure 4 presents the monthly responder rates, stratified by gender, of "Patient-reported global assessment of relief of IBS symptoms". In male patients, monthly responder rates were significantly higher in the ramosetron group than in the placebo group at all time-points ($p<0.001$ for all points). Although the proportion of monthly responder rates in female patients was also higher in the ramosetron group compared with the placebo group at each time-point, a significant effect was only observed at month 2 ($p=0.031$). The magnitude of efficacy was comparable to or higher in female patients relative to male patients.

Secondary efficacy evaluation: bowel-related functions. A significantly greater proportion of patients treated

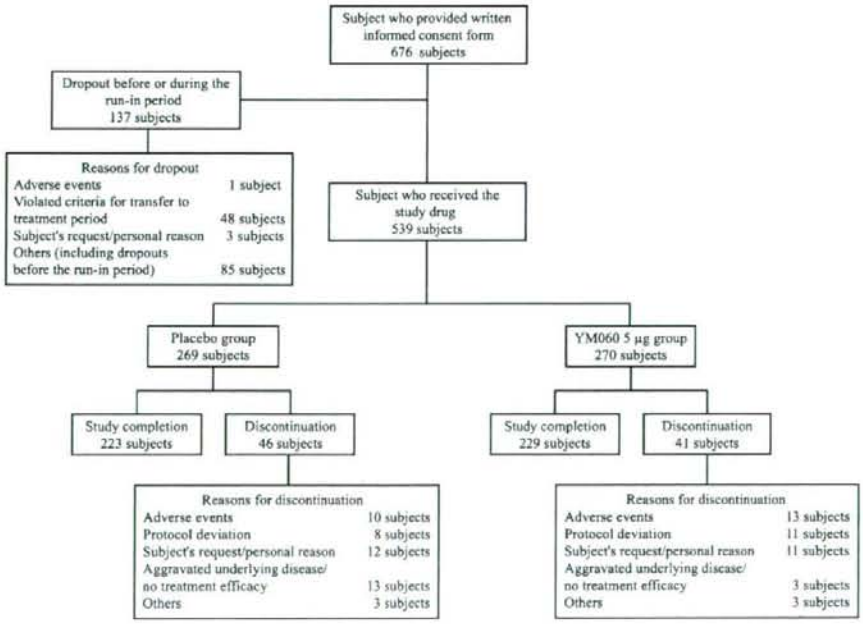


Figure 1. Flowchart of patient progression through the study. Reasons for dropouts and discontinuation from the study are indicated.

Table I. Demographics and baseline characteristics of participants.

Patient background			No. of subjects	Mean	SD	*t*-test
Age (years)	Total	Placebo	268	41.8	11.70	$t=1.089$, df $=535$
		5 μg	269	40.7	11.21	$p=0.276$
	Male	Placebo	226	41.9	11.38	$t=0.045$, df $=439$
		5 μg	215	41.8	10.84	$p=0.964$
	Female	Placebo	42	41.1	13.42	$t=1.958$, df $=94$
		5 μg	54	36.1	11.58	$p=0.053$
Duration of disease (months)	Total	Placebo	261	149.6	131.25	$t=0.337$, df $=522$
		5 μg	263	145.8	130.70	$p=0.736$
	Male	Placebo	219	155.4	128.76	$t=-0.207$, df $=427$
		5 μg	210	158.0	134.67	$p=0.836$
	Female	Placebo	42	119.7	141.41	$t=0.901$, df $=93$
		5 μg	53	97.3	100.88	$p=0.370$
Abdominal discomfort/pain	Total	Placebo	268	1.695	0.5991	$t=0.706$, df $=535$
		5 μg	269	1.658	0.6212	$p=0.480$
	Male	Placebo	226	1.677	0.6047	$t=0.893$, df $=439$
		5 μg	215	1.626	0.5991	$p=0.372$
	Female	Placebo	42	1.789	0.5660	$t=0.044$, df $=94$
		5 μg	54	1.783	0.6941	$p=0.965$
Stool form	Total	Placebo	267	5.323	0.6548	$t=0.094$, df $=529$
		5 μg	264	5.318	0.6430	$p=0.926$
	Male	Placebo	225	5.368	0.6413	$t=0.287$, df $=435$
		5 μg	212	5.351	0.6094	$p=0.774$
	Female	Placebo	42	5.084	0.6821	$t=-0.669$, df $=92$
		5 μg	52	5.184	0.7572	$p=0.505$
Stool frequency	Total	Placebo	268	2.752	1.3059	$t=0.511$, df $=535$
		5 μg	269	2.695	1.2726	$p=0.609$
	Male	Placebo	226	2.800	1.3152	$t=0.025$, df $=439$
		5 μg	215	2.797	1.3006	$p=0.980$
	Female	Placebo	42	2.493	1.2380	$t=0.862$, df $=94$
		5 μg	54	2.289	1.0721	$p=0.391$

Abdominal discomfort/pain: 0: None, 1: Mild, 2: Moderate, 3: Severe, 4: Intolerable.
Bristol Stool Form Scale: 1: Separate hard lumps like nuts (difficult to pass); 2: Sausage shaped but lumpy; 3: Like a sausage but with cracks on its surface; 4: Like a sausage or snake, smooth and soft; 5: Soft blots with clear-cut edges (passed easily); 6: Fluffy pieces with ragged edges, a mushy stool; 7: Watery, no solid pieces, entirely liquid.

with ramosetron reported adequate relief of abdominal discomfort and/or pain at the final point (46% versus 33%, $p=0.005$; Figure 5a). Ramosetron also contributed to significantly greater improvement of abnormal bowel habits compared with the placebo (44% versus 24%, $p<0.001$; Figure 5b). For each assessment, the effects were observed by month 1 and were sustained throughout the treatment.

For each symptom, Figure 6 shows the effects of ramosetron on stool form, stool frequency and bowel urgency. Ramosetron hardened stool form (Figure 6a), decreased stool frequency (Figure 6b) and increased the rate of days without bowel urgency (Figure 6c) compared with the placebo. Improvement was observed within the first week and was sustained throughout the treatment. The rate of days without the sensation of incomplete bowel movement was also decreased in the ramosetron group compared with the placebo group (data not shown).

Safety

All 539 patients who received the drug (269 who received placebos and 270 who received ramosetron) were evaluated for safety.

One hundred and sixty-three patients (60.37%) in the ramosetron group and 141 patients (52.42%) in the placebo group reported adverse events. Table II shows the adverse events occurring with a frequency greater than 2% in the ramosetron group. Hard stool was the most frequently reported adverse event in the ramosetron-treated group compared with the placebo group and occurred in 20 (7.41%) of 270 patients in the ramosetron group compared with 2 (0.74%) of 269 patients in the placebo group. No adverse events were classified as severe, and most events were classified as mild in the ramosetron-treated group. Drug-related adverse events with a frequency greater than 1% in the ramosetron group compared with the placebo group were abdominal discomfort, abdominal distension, constipation, hard stool and an increase in blood bilirubin. Other

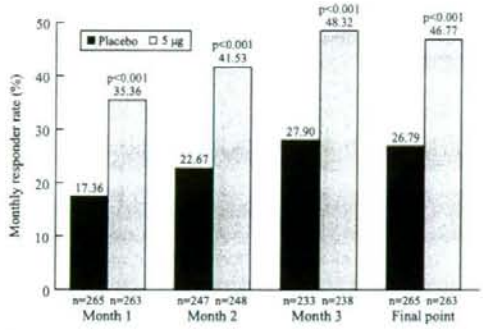


Figure 2. Monthly responder rate of "Patient-reported global assessment of relief of IBS symptoms". *P*-values were calculated using the χ^2 test.

adverse event profiles were similar in both groups. The incidence of drug-related adverse events was higher in females than in males. Drug-related adverse events for which the incidence in ramosetron-treated females was higher by 3% or more than that in males were abdominal distension, constipation, hard stool and a decrease in white blood cell count.

Thirteen patients (4.81%) in the ramosetron group and 10 patients (3.72%) in the placebo group discontinued the treatment because of adverse events. The adverse events associated with most of these discontinuations in ramosetron-treated patients were constipation (3 (1.11%)) and hard stool (3 (1.11%)).

Discussion

In this randomized, double-blind, placebo-controlled trial, the monthly responder rate based on "Patient-reported global assessment of relief of IBS symptoms" was higher in the ramosetron 5 μg group than in the placebo group, which verified the superiority of ramosetron 5 μg to placebos. It has been difficult to assess the effect of novel drugs for the treatment of IBS or other functional gastrointestinal disorders because of the multiplicity of symptoms and a high level of placebo effect. The Rome II working group guidelines recommended a patient-report outcome of the assessment as a primary end-point. Therefore, we used the "Patient-reported global assessment of relief of IBS symptoms" as a primary end-point. The primary end-point used in alosetron clinical trials was also the proportion of patients with adequate relief of IBS pain and discomfort for at least 2 weeks per month (defined as a monthly responder) for all 3 months [10–12]. Furthermore, in a recent report Camilleri et al. strongly insisted that global assessment should be a primary end-point in trials in IBS patients [21].

This study demonstrated the effects of ramosetron in both male and female Japanese patients with D-IBS meeting the Rome II diagnostic criteria. The statistically significant efficacy of ramosetron

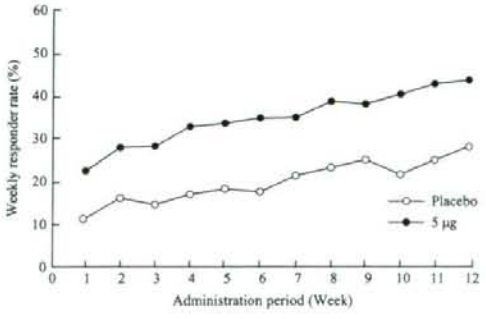


Figure 3. Weekly responder rate of "Patient-reported global assessment of relief of IBS symptoms". Weekly responders were patients whose scores were "Completely relieved" or "Considerably relieved".

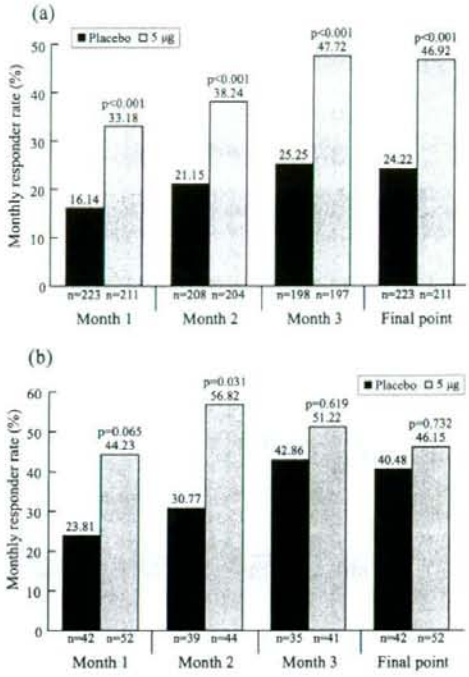


Figure 4. (a) Monthly responder rate of "Patient-reported global assessment of relief of IBS symptoms" in males. (b) Monthly responder rate of "Patient-reported global assessment of relief of IBS symptoms" in females. *P*-values were calculated using the χ^2 test.