The irritable bowel syndrome (IBS) is one of the most common disorders in gastroenterological practice [8, 26]. IBS is clinically characterized by stressful abdominal pain or discomfort, and altered bowel habits [7, 30]. Although the type and nature of altered bowel habits varies across the patients, abdominal pain has been demonstrated in the majority of patients suffering from IBS. Accumulated clinical data have shown that colonic sensory threshold to mechanical distention stimuli is markedly decreased in IBS patients [23, 31, 34], indicating a visceral hypersensitivity. Since alterations of the processing of sensory stimuli in the brain-gut axis are believed to play etiological or modulatory roles in the visceral hypersensitivity, the CNS sensation in IBS has been a focus of much attention [4–6]. Recent studies in humans employ non-invasive imaging technologies, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), to investigate the mechanisms and pathways involved in the CNS processing of sensory stimuli in normal and visceral pain states [2, 18, 29, 34]. These studies have generally revealed that multiple brain structures, such as the insular and cingulate cortices, prefrontal cortex and thalamus, are activated to a greater extent during the colonic distention stimuli in IBS patients compared to the normal subjects [16, 34]. The use of bio-imaging technologies has been further applied to determine the CNS mechanisms and sites of action of new drugs for treatment of abdominal pain in IBS [3, 20].

The behavioral, autonomic, and motor responses induced by colonic distention have been also observed in rats [21, 22, 24]. Recently, It has been reported that intra-luminal injection of 2,4,6-trinitrobenzene sulfonic acid (TNBS) into the proximal colon of rats resulted in sustained decrease in the sensory threshold of the distal colon where no inflammatory responses were observed [13, 25]. Based on the finding that TNBS-induced increase in colonic sensitivity lasted for 3 weeks [13], this experimental visceral hypersensitivity is considered to provide a valuable tool to investigate the pathophysiology of chronic functional gut disorders characterized by visceral hypersensitivity, including IBS. However, no evidence is available to date regarding the CNS activation in TNBS-sensitized rats. In order to confirm and further extend the usefulness of TNBS-induced colonic hypersensitivity as a model of IBS, it is necessary to determine whether it shows CNS activation that parallels the brain events observed in the clinical disease state. CNS activation potentially provides a more objective way to evaluate drug effects on pain perception.

Recent development of the microPET scanner has enabled application of PET technology to obtain three-dimensional molecular information within the living brain of rats and mice [14, 15, 19, 27], although most of the microPET experiments using the small laboratory animals have been conducted under anesthesia. In this study, we have developed a microPET protocol to measure 18F-fluorodeoxyglucose (18F-FDG) uptake as a quantitative marker of metabolic activity in the living rat brain. Using this protocol, we determined the changes in the brain activity in response to colonic distention in TNBS-sensitized rats. Further development of the microPET scanner has enabled application of PET technology to obtain three-dimensional molecular information within the living brain of rats and mice [14, 15, 19, 27], although most of the microPET experiments using the small laboratory animals have been conducted under anesthesia. In this study, we have developed a microPET protocol to measure 18F-fluorodeoxyglucose (18F-FDG) uptake as a quantitative marker of metabolic activity in the living rat brain. Using this protocol, we determined the changes in the brain activity in response to colonic distention in TNBS-sensitized rats. Further development of the microPET scanner has enabled application of PET technology to obtain three-dimensional molecular information within the living brain of rats and mice [14, 15, 19, 27], although most of the microPET experiments using the small laboratory animals have been conducted under anesthesia. In this study, we have developed a microPET protocol to measure 18F-fluorodeoxyglucose (18F-FDG) uptake as a quantitative marker of metabolic activity in the living rat brain. Using this protocol, we determined the changes in the brain activity in response to colonic distention in TNBS-sensitized rats. Further development of the microPET scanner has enabled application of PET technology to obtain three-dimensional molecular information within the living brain of rats and mice [14, 15, 19, 27], although most of the microPET experiments using the small laboratory animals have been conducted under anesthesia. In this study, we have developed a microPET protocol to measure 18F-fluorodeoxyglucose (18F-FDG) uptake as a quantitative marker of metabolic activity in the living rat brain. Using this protocol, we determined the changes in the brain activity in response to colonic distention in TNBS-sensitized rats. Further development of the microPET scanner has enabled application of PET technology to obtain three-dimensional molecular information within the living brain of rats and mice [14, 15, 19, 27], although most of the microPET experiments using the small laboratory animals have been conducted under anesthesia. In this study, we have developed a microPET protocol to measure 18F-fluorodeoxyglucose (18F-FDG) uptake as a quantitative marker of metabolic activity in the living rat brain. Using this protocol, we determined the changes in the brain activity in response to colonic distention in TNBS-sensitized rats.
In an attempt to explore potential value of the microPET brain image as a biological marker of visceral pain, additional experiments were conducted to investigate and compare the inhibitory effects of morphine on visceral nociception and brain activation elicited by colonic distention in TNBS-sensitized rats.

MATERIALS AND METHODS

**Animals:** Male Sprague-Dawley rats (240–270 g body weight, Charles River Inc.) were kept under conditions of constant temperature (21 ± 2°C) and humidity (55 ± 10%) with a 12-hr light/dark conditions with free access to normal laboratory chow and tap water. All procedures employed in the experiments were approved by the institutional Animal Ethics Committees according to the Laboratory Animal Welfare guidelines.

**TNBS-induced hypersensitivity:** After 16–18 hr fasting, the animals were anesthetized by combined intramuscular administration of ketamine (40 mg/kg) and xylazine (6 mg/kg), abdominal laparotomy was made for injection of TNBS (50 mg/1.5m/kg) into the proximal colon (1 cm distal from the cecum). The sham-operated rats were prepared with the same surgical procedure, but received vehicle alone instead of TNBS. Measurement of visceral pain threshold was carried out in unanesthetized rats as described previously [13, 25]. In short, a 5-cm latex balloon (Okamoto, Japan) was inserted through the anus and placed in the distal colon at 5 cm from the anus. After 30-min acclimation, the balloon was progressively inflated from 0 to 70 mmHg, by 5 mmHg increment every 30 sec using the electronic barostat (G&J, Canada). The distention procedure was repeated twice with 10 min interval, i.e. the first stimuli as the preliminary conditioning one and the second distention to record the pain threshold. The pain threshold was defined as the pressure inducing the characteristic painful behaviors (i.e., abdominal cramp), corresponding to the repeated waves of contraction of oblique musculature with inward turning of the hind limb, or to hump-backed position, or to squashing of the lower abdomen against the floor [32]. In some experiments, animals were treated with subcutaneous morphine (125 and 375 µg/kg) at 20 min before 18F-FDG injection, and repeated three times with 1 min interval in the uptake period. The animals were then put on a heated (37°C) water blanket imaging bed on the Focus 220 PET scanner (Siemens-CTI-Concorde Microsystems Inc, Knoxville, Tennessee) under anesthesia with 2% isoflurane inhalation, and the head was centered in the scanner field of view and fixed in place by tooth bar and ear canal holders. Systemic imaging was conducted in the coronal plane from the frontal cortex to the occipital cortex. The data acquisition was started at 40 min post 18F-FDG, and continued for 20 min. In some experiments, rats had a subcutaneous injection of morphine (125 and 375 µg/kg, s.c.) at 20 min before 18F-FDG injection.

The PET images were reconstructed into a 128 × 128 × 95 volume with in-plane pixel dimension of 0.6 mm and slice thickness of 0.796 mm. After corrections for detector normalization, decay, attenuation and scatter, the pixel values on the image are scaled by the injected activity and the body weight of each individual animal, resulting in the Standardized Uptake Values (SUV) image which is a body size-independent index to quantify tissue uptake of 18F-FDG. Reconstructed images were co-registered and mapped to a stereotaxic rat brain atlas for subsequent identification of tissue regions of interest (ROIs) using an in-house developed image co-registration software based on mutual information algorithm. Statistical parametric mapping (SPM) analysis was performed to detect any brain pixels that have statistically significant differences between treatments using the AFNI software developed by the NIH [11]. For ROIs showing significant differences in the SPM analysis, maximum SUV value was extracted in each rat, and one-way ANOVA analysis followed by pair-wise multiple comparisons (Student-Newman-Keuls Method) was performed to detect the difference of mean maximum SUV values between groups using Sigmastat version 2.03 software (SPSS Inc, Chicago, IL).

**Compounds:** 2,4,6-trinitrobenzene sulfonic acid was obtained from Fluka (Buchs, Switzerland) and dissolved in 30% ethanol at 33 mg/ml for intra-colonic injection. Morphine sulfate was purchased from Merck & Co. Inc (Rahway, NJ) and dissolved in physiological saline for subcutaneous injection. 18F-fluorodeoxyglucose was purchased from Eastern Isotopes Inc. (Romeoville, IL). Ketamine/xylazine solution was purchased from Sigma Aldrich Co. (St. Louis, MO).

RESULTS

**Time-course of TNBS-induced colonic hypersensitivity:** In order to determine the time-course of development of visceral hypersensitivity and the day when the difference between TNBS and sham groups was greatest, the alterations in colonic pain threshold to mechanical distention were studied for 21 days after the surgery. On day 3, both sham-operated and TNBS-sensitized animals demonstrated marked decreases in the threshold distention required to elicit behavioral signs of pain, and there was no significant
difference in the pain threshold between the two groups (Fig. 1). On day 5, the colonic pain threshold of TNBS-sensitized rats remained about half of the pre-surgery normal value, whereas the sham-operated control rats showed almost complete recovery. The TNBS-induced colonic hypersensitivity achieved maximal magnitude on day 7, which then subsequently subsided in the following week, but remained at significantly lower level even on day 14 relative to the sham-operated control values. On day 7, all the TNBS-sensitized animals showed the characteristic pain behaviors in response to colonic distention stimuli at 35 mm Hg and below, but none of sham-operated rats showed any behavioral changes in response to the normally non-painful distention stimuli.

Brain imaging by microPET: Based on the findings in the study of the time-course of development of hypersensitivity, we carried out microPET imaging experiments on day 7 post-surgery, using colonic distention stimuli by repeated progressive balloon inflation (0 to 35 mmHg × 3) that elicited the visceral pain in TNBS-sensitized rats, but not in sham-operated animals. Using $^{18}$F-FDG as a tracer, microPET had sufficient resolution to measure glucose metabolism in the rat brain, and the scans presented clear images of brain structures. Systemic brain imaging conducted in the normal condition without colonic distention stimuli demonstrated that no obvious differences were detected in the brain PET images between the sham-operated and TNBS-sensitized rats (data not shown). Also, no significant change in the brain $^{18}$F-FDG uptake was produced in the sham animals subjected to the low pressure (<35 mmHg) stimuli (data not shown). Figure 2 illustrates the outcomes of the SPM analysis of the differences in the brain $^{18}$F-FDG uptake between TNBS-sensitized rats subjected to low pressure colonic distention stimuli (<35 mmHg) and sham-operated control animals in the normal condition without colonic distention stimuli. In TNBS-sensitized group subjected to colonic distention, tissue uptake of $^{18}$F-FDG was significantly increased in the thalamus (Fig. 2A) and sensory cortex I (Fig. 2B) relative to sham group without distention stimuli. No significant changes were detected in other regions of interest, such as cingulate and insular cortices.

Effects of morphine on the brain FDG uptake and colonic

![Thalamus](A)  
TNBS with Colonic Distention vs. Sham without Colonic Distention

![Sensory Cortex I](B)  
TNBS with Colonic Distention vs. Sham without Colonic Distention

Fig. 1. Time course of the changes in colonic pain threshold to mechanical distention in TNBS-treated rats. The threshold balloon pressure (mmHg) required for induction of an abdominal cramp in each group was determined at 0, 3, 5, 7, 10, 14, 18 and 21 days after TNBS injection into the proximal colon. Each point represents the median value of 6–8 rats, and the vertical bars express the first (lower) and third (upper) quartiles that indicate a range of median values calculated by Prism Software (GraphPad, CA, U.S.A.). The statistical analysis was carried out using Kruskal-Wallis testing followed by individual Mann-Whitney U-test. * P<0.05, ** P<0.01 vs. sham rats.

Fig. 2. Statistical parametric mapping (SPM) analysis of the differences in the brain $^{18}$F-FDG uptake between TNBS-sensitized rats subjected to low pressure colonic distention (0–35 mmHg) and sham-operated animals in the normal condition without colonic distention stimuli. Color scale indicates percent increase (red) and decrease (blue) in the mean value of brain $^{18}$F-FDG uptake of the TNBS-sensitized, colonic distention group (n=6) relative to sham-operated non-distention group (n=6). A: thalamus, B: sensory cortex I.
pain threshold: To investigate whether the regional brain activation detected by microPET was a pain-mediated phenomenon, we examined effects of morphine on brain 18F-FDG uptake and colonic pain threshold in TNBS-sensitized rats. In the behavioral pharmacology study, the lower dose (125 µg/kg, s.c.) of morphine produced only marginal, non-significant effect on the colonic pain threshold (Fig. 3). At the higher dose (375 µg/ kg, s.c.), however, the µ-opioid receptor agonist showed potent antinociceptive effects against visceral pain induced by colonic distention in the TNBS-sensitized rats, as evidenced by a reversal of the pain threshold nearly to the normal control level. In the micro-PET study, the lower dose of morphine was without significant effect on the brain 18F-FDG uptake that was elevated by the low pressure colonic distention (0 to 35 mmHg) in TNBS-sensitized rats. As shown in Fig. 4, however, pretreatment with the higher dose of morphine almost completely suppressed the distention-induced elevation of 18F-FDG uptake in the thalamus and sensory cortex I of rats with sensitized colon. Further analysis of the maximum SUV values also determined that the effect of the higher dose of morphine on the tissue 18F-FDG uptake of the brain regions was of statistical significance (P<0.01, Fig. 5).

DISCUSSION

Advanced imaging technologies, such as fMRI and PET, have enabled noninvasive investigation of somatic and visceral sensation including pain perception in humans [2, 9, 18, 29]. In the present study, microPET technology was employed to investigate the regional brain activation induced by colonic distention in rats in which visceral hypersensitivity had been induced by intra-colonic injection of TNBS. Anesthesia depresses a variety of physiological responses, including neuronal activation and metabolism. Our microPET protocol was designed to minimize the effect of anesthesia by keeping animals conscious during a 30-min
18F-FDG uptake period while colonic distention stimulus was repeated. Using this protocol, microPET had sufficient resolution to measure glucose metabolism in the rat brain, and the scans provided clear images of several brain structures, including the thalamus, striatum and cerebellum.

In sham-operated control rats, neither pain-induced behaviors nor elevated brain 18F-FDG uptake were caused by low pressure colonic distention (35 mmHg and below) on Day 7 post-surgery. This finding is in general agreement with that of a recent fMRI study [17], in which the colonic pain threshold and regional cerebral activation were determined using non-sensitized, normal rats under anesthesia. In the fMRI study, distention at 40 mmHg produced no cerebral activation, while significant activation in many brain structures was observed following distention stimuli of 60 mmHg and greater. Our behavioral pharmacology data also showed that median value of colonic sensory threshold of sham-operated control rats was 50 (range=42.5–57.5) mmHg. Thus the colonic pain threshold of normal rats is most likely to be in the range between 40 and 60 mmHg.

A significant, sustained decrease in the colonic pain threshold to mechanical distention was observed in TNBS-treated rats, indicating development of a colonic hypersensitivity. On day 7 post-TNBS, the colonic hypersensitivity became fully established, and microPET revealed that 18F-FDG uptake was significantly elevated in the thalamus and sensory cortex I in response to the low pressure colonic distention.

Numerous studies of somatic pain have identified a series of cerebral regions involved in the processing of somatic pain, including anterior cingulate cortex, prefrontal cortex, insular cortex and thalamus [10, 12]. These studies pointed to the thalamus as a relay center, connecting the afferent signals to the higher centers such as the cingulate and insular cortices. Recent imaging studies of visceral pain have generally demonstrated that these pain loci are also important in the CNS processing of visceral sensation in IBS patients [2, 18, 29, 34] which is consistent with the present observations. A previous fMRI study [17] using non-sensitized, normal rats has shown that the amygdala, hypothalamus, thalamus, cerebellum and hippocampus were significantly activated by relatively higher colonic balloon distention, i.e., 60 mmHg and above, under anesthesia. Differences between the previous fMRI study and our microPET study could be attributed to the fact that non-painful low pressure distention was used in this study, to differences in detection sensitivities between fMRI and microPET technologies, or to differences in the timing of the measurements. In the previous study, responses were measured during stimulation, whereas in our study, the stimuli were applied immediately prior to the imaging.

Our microPET data also demonstrated that treatment with an analgesic dose of morphine (375 µg/kg, s.c.) resulted in a complete suppression of the regional brain activation in TNBS-sensitized rats. It is extensively acknowledged that morphine produces a potent antinociceptive activity via spi-
nal and supra-spinal pathways to inhibit somatic pain and nociceptive reflexes [33]. Therefore it is reasonable to consider that the regional brain activation detected by microPET is a pain-mediated central phenomenon. On the other hand, no significant changes occurred in the brain 18F-FDG uptake of the animals treated with the lower dose of morphine (125 µg/kg, s.c.) that produced only marginal, non-significant effect on visceral pain induced by colonic distention. Thus the morphine-induced inhibition of the visceral nociception appears to parallel the decrease in the brain 18F-FDG uptake, suggesting that PET would provide an objective biomarker for assessing the pharmacological effects of anti-hyperalgesic agents.

The mechanisms by which application of TNBS to the proximal colon elicits hypersensitivity to distal colon distension are unknown. TNBS, administered into the proximal colon, produced neither mucosal lesions nor inflammatory cell infiltration in the distal colon, and the tissue myeloperoxidase activity in the distal colon was very low, suggesting that the altered sensory processing was not directly mediated by tissue damage or inflammatory mediators [13, 25]. Sengupta et al. have reported that there were a greater number and activity of the pelvic nerve fibers of TNBS-treated rat colon in response to mechanical distension [28]. Recently, we have found in rats that an injection of TNBS into the proximal colon resulted in the prominent mast cell infiltration with enhanced spontaneous mediator release, and that TNBS-induced visceral hypersensitivity was significantly suppressed by a mast cell stabilizer dexametazanazole [25]. Since mast cell mediators, such as histamine and tryptase, can excite enteric sensory nerves [1], it is conceivable that an enhanced spontaneous release of the excitatory mediators is responsible, at least partly, for the increased colonic sensitivity to distension stimuli in TNBS-treated rats. Transient decrease in the colonic sensitivity observed in the sham-operated rats on Day 3 is unknown, but may be ascribed to the post-surgery stress.

In summary, our microPET study demonstrates for the first time that regional brain activation is associated with altered processing of colonic sensory stimulus in TNBS-treated rats. This provides an objective method to evaluate effects of drugs that can intervene in pain signaling to the cortex. It avoids the ambiguity of other methods that measure surrogates of pain, such as blood pressure changes and abdominal wall contractions.

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