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Monoamine system disruption induces functional somatic syndromes associated symptomatology in mice

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ABSTRACT

Functional somatic syndromes (FSS), a clinical condition manifesting a variety of unexplained somatic symptoms, has been proposed as an inclusive nosology encompassing individual syndromes such as fibromyalgia syndrome and irritable bowel syndrome. Accumulating evidence suggests that disturbance of the endogenous monoamine system could be involved in the aetiology of FSS. Therefore, the purpose of present study was to investigate whether the disturbance of the monoamine system would cause FSS-associated symptomatology in mice. The optimal dose of reserpine, an inducer of endogenous monoamines reduction, was first explored in mice. General body condition (body weight, rectal temperature, and ptosis) and FSS-associated symptomatology (paw withdrawal threshold, small intestinal transit, and locomotor activity) were measured. The concentration of monoamines was measured in central and peripheral tissues. Mice dosed with reserpine (0.25 mg/kg s.c., once daily for 3 consecutive days) exhibited a decrease in paw withdrawal threshold, delay in small intestinal transit, and reduction of locomotor activity without deterioration of general body condition on day 5 after the first reserpine injection. The concentration of monoamines was decreased in the central nervous system and skeletal muscle, but not in the small intestine. A reserpine dose of 0.5 mg/kg or more caused deterioration of general body condition. In conclusion, the optimal protocol of reserpine treatment for inducing pain symptom without deterioration of general physical condition is 0.25 mg/kg s.c., once daily for 3 consecutive days in mice. This protocol causes not only pain but also FSS-associated symptomatology which are associated with disruption of the endogenous monoamine system. The reserpine-treated animal may be useful for the research of not only fibromyalgia syndrome but also FSS, especially for the research focusing on the hypothesis that FSS is associated with the disturbance of endogenous monoamine system.

1. Introduction

Multiple clinical conditions with a variety of somatic symptoms remain unexplained by structural or biochemical anomalies [1,2]. Patients with such symptoms are often diagnosed as individual syndromes including fibromyalgia syndrome (FMS) [3], irritable bowel syndrome (IBS) [4], and chronic fatigue syndrome (CFS) [5]. Although the predominant symptoms of FMS, IBS, and CFS are widespread musculoskeletal pain, recurrent abdominal pain/discomfort, and chronic fatigue, respectively, such individual syndromes have a high degree of similarity and co-occurrence. The same patient often meets the diagnostic criteria for more than one individual syndrome. In view of the substantial overlaps amongst these individual syndromes, functional somatic syndromes (FSS), an inclusive nosology encompassing the individual syndromes, has been proposed [6–10]. FSS is highly prevalent [11–14] and has a large negative impact on both individual patients and society [15–18]. It has been shown that FSS is as debilitating as well-established degenerative diseases, such as rheumatoid arthritis, in leading to considerable functional limitations and loss of employment [19]. Existing therapies do not provide adequate relief from symptoms [15,17]. Thus, characterization of FSS pathophysiology is an urgent issue. Given FSS is currently a serious health issue, European Network on Somatic Symptom has recently recommended the core outcome domains for clinical trials in patients with functional somatic syndromes [14].

Although pathogenesis of FSS is unclear, accumulating evidence suggests that endogenous monoamine system is involved in its aetiology. Several gene polymorphisms associated with the monoamine system are suggested to be possible risk factors for FMS [20], IBS [21], and CFS [22]. Drugs that modulate the monoamine system, including serotonin, norepinephrine reuptake inhibitors, and atypical antipsychotics, attenuate FSS-associated symptoms [23]. Responses of the monoamine system are attenuated in patients with FMS [24] and CFS [25,26].

Animal models of disease are a valuable tool in the characterization of pathophysiology. To our knowledge, no study has been conducted to develop an animal model which exhibits FSS-associated symptomatology, although animal models of individual syndromes such as FMS [27,28] and IBS [29] are established. Based on the critical role of the monoamine system, we postulate that the disturbance of the

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monoamine system triggers the development of FSS. It was reported that the reduction of monoamines by reserpine, an inhibitor of storage of monoamines in the synaptic vesicles, induced FMS-like pain in rats [30–37]. Given that the symptoms overlap across individual syndromes (FMS, IBS, and CFS), we conceived that an animal model of an individual syndrome such as FMS would possibly manifest the symptoms dominant in other syndromes, i.e. the animal model of one syndrome would potentially exhibit FSS-associated symptomatology. In the present study, we investigated whether FSS-associated symptomatology is triggered by reserpine treatment in mice. Because the protocol of reserpine treatment for inducing pain symptom in mice varies in previous studies [38–42], the optimal protocol of reserpine treatment was first explored, then, FSS-associated symptoms and the concentration of monoamines in the central and peripheral tissues were measured.

2. Materials and methods animals

2.1. Animals

Animal experiments and care were conducted in accordance with the institutional guideline that follows Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science, and Technology (Notice No. 71, Japan, 2006). All experimental procedures were approved by the institutional animal care and use committee of Aomori university (Aomori, Japan). Animal studies follow the ARRIVE guidelines [43]. All efforts were taken to minimize the number and suffering of animals used.

Male ICR (24–33 g) and C57BL/6J (21–24 g) mice were purchased from CLEA Japan, Inc., (Tokyo, Japan). Animals were group-housed (eight per cage) with wood chip bedding under a 12 h light/dark cycle (lights on: 08:00–20:00) at a controlled temperature (23 ± 1 °C) and humidity (55 ± 10%) with free access to standard laboratory chow and water. All tests were conducted during the light phase. Animals were allocated to experimental groups using software (Simple Grouping; H&T Co., Osaka Japan) to ensure that all groups were comparable in terms of body weight. Animals were deprived of food for 18 h only prior to the measurement of small intestinal transit. All measurements were conducted by experimenters blinded to the treatment (vehicle or reserpine).

C57BL/6J strain was used only in the experiment for examining a strain difference in the tolerability to reserpine. ICR strain was used in all the experiments.

2.2. Drugs

L-(−)-norepinephrine-(+)-bitartrate and 3-hydroxytyramine hydrochloride (dopamine) were purchased from Merck Millipore (Darmstadt, Germany) and Tokyo Chemical Industry (Tokyo, Japan), respectively. Serotonin (5-hydroxytryptamine)-creatinine sulfate monohydrate was obtained from Wako Pure Chemical Industries (Osaka, Japan). Reserpine and carmine were purchased from Nacalai Tesque (Kyoto, Japan) and Sigma–Aldrich (St. Louis, MO), respectively. Reserpine was dissolved in glacial acetic acid, and diluted to a final concentration of 0.5% acetic acid with distilled water. Reserpine was then injected s.c. in a volume of 10 ml/kg. Doses refer to the free bases. Exploration of optimal dosing regimen of reserpine was conducted in the present study. The regimen, 1 mg/kg s.c., once daily for 3 consecutive days, was first applied according to our previous study in rats [30,31]. The regimen, 0.25 mg/kg s.c., once daily for 3 consecutive days, was consequently identified as an optimal one to induce FSS-associated symptoms in mice.

2.3. Paw withdrawal threshold

The threshold of paw withdrawal response to tactile stimuli was measured using von Frey filaments (Ugo Basile, Varese, Italy). Each mouse was placed in a transparent test cage (width: 100 mm, depth: 100 mm, height: 140 mm) with a perforated metal platform. Von Frey filaments (0.02, 0.07, 0.17, 0.41, 1.2, 2.0, 3.6, and 5.4 g) were applied to the middle plantar surface of the right hind paw. Paw withdrawal response to a filament stimulus was defined as a positive response, while lack of response within 3 s was defined as a negative response. The 0.41 g force filament was applied first. When a positive response to a given filament was obtained, the filament of the next smaller force was applied. When a negative response was obtained, the filament of the next larger force was applied. The test was repeated until four responses were collected following the first change in response. The 50% paw withdrawal threshold was determined according to the up-down method [44]. Baseline (day 0) paw withdrawal thresholds were measured in 24 ICR mice before the first injection of reserpine. The animals were then divided into three groups (n = 8/group). Each group of animals received a subcutaneous injection of reserpine at a dose of 0 (vehicle), 0.125, or 0.25 mg/kg once daily for 3 consecutive days. The paw withdrawal thresholds were again measured on days 1, 2, 3, 5, 7, and 9 after the first injection of reserpine.

2.4. Small intestinal transit

Small intestinal transit was measured according to the procedure described by Kimball et al. [45]. Carmine, a dye, was employed as a marker based on its property that it is not absorbed from the lumen of the gut. A 6% carmine suspension in 0.5% methylcellulose was prepared and administered orally with cannula in a volume of 0.3 ml per animal. The animal was sacrificed by cervical dislocation 20 min following administration. The abdomen was incised, and the intestine was removed from the pyloric junction to the caecal end. Both the distance travelled by the head of the marker and the total length of the small intestine were measured. Small intestinal transit was expressed as a percentage of the distance travelled by the head of the marker relative to the total length of the small intestine. Animals were divided into two groups (n = 8/group). Each group of animals received a subcutaneous injection of vehicle or reserpine at 0.25 mg/kg once daily for 3 consecutive days. Small intestinal transit was measured on days 5 and 7 after the first reserpine injection. Separate set of 16 ICR mice was used on each measurement day (i.e. days 5 and 7).

2.5. Locomotor activity

Mice were acclimated to the test room for 1 h prior to the beginning of the experiment. Locomotor activity was measured using an animal movement analysis system (SCANET MV-40; Melquest, Toyama, Japan), which monitors the horizontal and vertical activities of animals. Horizontal activity was expressed as number of beams crossed, and vertical activity corresponded to the number of rearings. All activity in a transparent plastic cage (438 mm × 438 mm × 295 mm) was automatically recorded for 10 min following the placement of the animal in the corner of the cage. Animals were divided into two groups (n = 8/group). Each group of animals received a subcutaneous injection of vehicle or reserpine at 0.25 mg/kg once daily for 3 consecutive days. Locomotor activity was measured on days 5, 7 and 9 after the first reserpine injection. Separate set of 16 ICR mice was used on each measurement day (i.e. days 5, 7 and 9).

2.6. Catalepsy

Catalepsy test was conducted with a horizontal bar (diameter = 0.5 cm) elevated 4.5 cm from the floor. Animal was placed with both forelegs on the bar. The catalepsy time, i.e. the time (latency)