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Somatic pain sensitivity during formation and healing of acetic acid-induced gastric ulcers in conscious rats

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Abstract

A classical feature of visceral pain is its referring to somatic locations. Gastric ulcer is a source of visceral pain. In the present study we investigated whether gastric ulcers may trigger the changes in somatic nociception. For this aim somatic pain sensitivity was estimated under conditions of gastric ulcer development and healing. Gastric ulcers were induced by luminal application of 60% acetic acid under surgical conditions. Control rats were subjected to the same surgical procedure, but with the application of saline instead of the acid. Somatic pain sensitivity (tail flick latency), plasma corticosterone level, adrenal and thymus weight were investigated under conditions of the formation and the healing of gastric ulcers. The application of the acid resulted in the formation of kissing gastric ulcers, the increase of somatic pain sensitivity (the decrease of tail flick latency) as well as the appearance of typical signs of chronic stress: long-lasting increase of plasma corticosterone level, adrenal gland hypertrophy and thymus gland involution. Natural healing of gastric ulcers was accompanied by restoration of pain sensitivity as well as attenuation of the signs of chronic stress. Delay of ulcer healing by the daily indomethacin administration (2 mg/kg, s.c.) prevented the restoration of somatic pain sensitivity. The results suggest that chronic gastric ulcers may trigger somatic hypersensitivity.

Keywords: Visceral and somatic pain sensitivity; Tail flick latency; Acetic acid-induced kissing gastric ulcers; Corticosterone; Conscious rats

1. Introduction

Although visceral pain is the most common form of pain produced by disease and one of the most frequent reasons for patients to seek medical treatment, nevertheless our knowledge about the basic mechanisms of pain derives from experimental studies of somatic nociception.

To distinguish from somatic pain, visceral pain is diffuse and poorly localized. A classical feature of visceral pain is that the sufferer perceives the pain as arising from somatic sites distant from the area of visceral pain source. This phenomenon of referred pain attenuates a real source of pain and complicates a diagnostic of disorders (Mayer and Gebhart, 1994; Cervero and Laird, 1999; Joshi and Gebhart, 2000). Referred pain/hyperalgesia was demonstrated in both clinical and animal studies using various diseases or animal models (Giamberardino et al., 1995; Wesselmann et al., 1998; Jaggar et al., 1999; Farquhar-Smith and Rice, 2001; Winston et al., 2005).

Gastrointestinal disorders became a serious problem of human population. Upper gastrointestinal bleeding continues to be wide spread despite major advances in endoscopy, pharmacology and surgery. Patients with various gastrointestinal disorders, including both gastric ulcer diseases and functional bowel disorders, suffer from gastrointestinal pain and discomfort. It is reasonable to propose that gastrointestinal pain as visceral pain triggers changes in somatic pain sensitivity. Although there is information regarding the effects of gastrointestinal inflammation of various aetiologies on somatic pain nociception, there are no studies about influence of visceral pain induced by gastric ulcers on somatic nociception. Moreover, it is not possible to definitely say about the character of influence of gastroin-

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testinal inflammation on somatic nociception. In human it was shown that chronic inflammation in gastrointestinal tract may induce both hyperalgesia (Verne et al., 2001, 2003; Bouin et al., 2001) and hypoalgesia (Cook et al., 1987; Accarino et al., 1995; Chang et al., 2000) in somatic fields. Conflicting data regarding this point were also obtained in animal experiments. Both somatic hyperalgesia (Kawao et al., 2004; Laird et al., 2001; Millecamps et al., 2004) and somatic hypoalgesia (Traub and Wang, 2004) have been demonstrated in rats and mouse with gastrointestinal inflammation. The discrepancy of the results of basic clinical researches and as well as human studies may be due to various reasons, including an evaluation of somatic nociception using different pain stimulus and in different somatic fields. The estimation of somatic pain sensitivity in different periods of gastrointestinal diseases may also contribute to the discrepancy.

The present study was performed to elucidate whether gastric ulcers may trigger the changes in somatic pain sensitivity. For this aim somatic pain sensitivity was estimated under conditions of gastric ulcer development and their natural or delay healing.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (Stolbovoe, Moscow, Russia), weighing about 250 g, were used. Five animals were housed per cage, and animals were acclimatized to standard laboratory conditions (12:12 h light–dark cycle, temperature 20 ± 1 °C, free access to food and water) for 7 days before use. The animals were kept in cages with raised mesh bottoms to prevent coprophagy and deprived of food but allowed free access to tap water for 18 h before the experiment. The experiments were performed according to the Helsinki agreement on the guiding principles for research involving animals and human beings. The experiments were approved the institutional scientific council.

2.2. Induction of gastric ulcers

Gastric ulcers were induced by liminal application of acetic acid solution, accordingly to Okabe method (Tsukimi et al., 2001) with slight modification as we published previously (Filaretova et al., 2002). Briefly, the animals were fasted for 18 h, anesthetized with ether, and operated upon to open their abdomens. Each stomach was exposed and the centers of the corpus area were clamped with ring forceps (ID 5 mm). A 60% acetic acid solution was ingested into the luminal side at the clamped portion. Forty-five seconds later, the acid was removed and the abdomen was closed. Control rats were subjected to the same surgical procedure, but with the application of saline instead of the acid.

In the end of experiments, the stomachs were removed, inflated by injecting 10 ml of saline. Thirty minutes later, each stomach was opened along the greater curvature. Then, the area (mm^2) of each ulcer developed in the corpus mucosa was measured under a dissecting microscope with a square grid (× 10). By this method, ulcers were produced both in anterior and posterior gastric walls (kissing gastric ulcers), the sum of both ulcerated areas were calculated and expressed as ulcer size per animal (Tsukimi et al., 2001).

Accordingly our previous results (Filaretova et al., 2002) at the 4th day after application of the 60% acetic acid solution onto the gastric mucosa (the day of the application was considered as the 1st day), penetrating ulcers developed in the gastric mucosa with 100% incidence and with average ulcerated area about 40 mm^2. The chronic ulcers healed enough fast, with rapid healing rate (about 5 mm^2/day) in the first 7 days after ulceration (Filaretova et al., 2002). By these reasons, in the present study we defined the 4th day (day 4) as the day of ulceration and the 11th day (day 11) as the day of healing.

2.3. Pain test

Tail-flick (TF) latency assay (D'Amour and Smith, 1941) was used for measurement of somatic pain sensitivity in conscious rats. Pain reaction was induced by thermal stimulation of a tail by using light beam analgesia meter. During the pain testing the rats were placed into the dark tube with their tail protruding and by this way were lightly restrained for the period of the test. The tube, in turn, was placed on the platform and the tail was positioned along special groove so that the radiant heat was focused onto the ventral surface of the tail at 6–7 cm proximal to the tip. The intensity of the heat source was adjusted to produce a baseline response time of 8–10 s. To prevent tissue injury, a cutoff time of 20 s was chosen. Results for each animal were the median of 3 trials. The TF latency was measured at 60 s intervals starting with the skin of the distal part of the tail-tip and continuing proximally from the preceding spot, similarly as in other studies (Whiteside et al., 2004; Young and Shalchi, 2005).

2.4. Experimental protocol

To adapt the animals to the experimental environment on the measurement of pain sensitivity, they were placed into the dark tube for 3 min once daily for 1 week before the beginning of experiment. After the adaptation, TF latency was measured (baseline TF latency) and then the rats were fasted for 18 h before the surgical procedure. The acetic acid solution or saline was ingested into the stomach under the surgical manipulation, and the pain sensitivity was tested 2.5 h after the surgery. This day was defined as day 1 of ulcer formation. The animals were fed normally from the next morning. All these steps were common for the Experiments 1 and 2.