Elevation of histidine decarboxylase activity in skeletal muscles and stomach in mice by stress and exercise

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Elevation of histidine decarboxylase activity in skeletal muscles and stomach in mice by stress and exercise. Am J Physiol Regulatory Integrative Comp Physiol 279: R2042–R2047, 2000.—The effects of different types of stress (water bathing, cold, restraint, and prolonged walking) on histidine decarboxylase (HDC) activity in masseter, quadriceps femoris, and pectoralis superficial muscles, and in the stomach were examined in mice. All of these stresses elevated gastric HDC activity. Although water bathing, in which muscle activity was slight, was sufficiently stressful to produce gastric hemorrhage and to increase gastric HDC activity, it produced no detectable elevation of HDC activity in any of the muscles examined. The other stresses all elevated HDC activity in all three muscles. We devised two methods of restraint, one accompanied by mastication and the other not. The former elevated HDC activity in the masseter muscle, but the latter did not. These results suggest that 1) HDC activity in the stomach is an index of responses to stress, 2) the elevation of HDC activity in skeletal muscles during stress is induced partly or wholly by muscle activity and/or muscle tension, and 3) stress itself does not always induce an elevation of HDC activity in skeletal muscles.

Histamine; masseter muscle; mastication; temporomandibular disorders

MUSCLE FATIGUE due to unaccustomed hard and/or prolonged physical exercise may lead to both pain and stiffness in skeletal muscles. However, the underlying molecular mechanisms have not been established. The pain and stiffness in craniofacial muscles seen in temporomandibular disorders (TMD) are also typical symptoms that are thought to be due to muscle fatigue resulting from abnormal masticatory exercise (such as bruxism) or from improper occlusion.

As to the relationship between stress and muscle fatigue, many studies have been carried out on TMD. With regard to the etiology of TMD, Haber et al. (14) presented the hypothesis that psychological stress or a stress-related emotional response might have a primary role in its development. This stress model hypothesizes that excessive psychological stress induces masticatory muscle hyperactivity that is expressed as abnormal muscle activities such as teeth clenching and/or grinding (or bruxism), causing eventual pain in muscle and joint, joint noise, and limitation of jaw motion. This model suggests that such stress is a common underlying etiological factor for TMD. Clenching and grinding easily damages masticatory muscles (6, 27), and healing of damaged joints is slow (17). Therefore, the stress model leads us to the idea that short-term psychological stress might induce muscular pain, whereas chronic stress would be associated with combined muscle and joint pain or just joint pain. Although this stress model has not been supported by some other investigators (18–20), the importance of psychological stress as an etiological agent has been repeatedly emphasized with regard to the development of muscular pain both in TMD (13, 15, 21, 23) and in shoulder and neck pain (28).

Histamine, a classical and important mediator of inflammation, produces pain as well as dilatation of precapillary arterioles and enhanced capillary permeability (2, 3, 12). It is pooled in large quantities in mast cells in the tissues and in basophils in the blood (2, 12), and there is an increase in blood histamine during hard physical exercise (7). Histamine is supposed to be released from mast cells during muscle damage, and it may then induce pain and/or vasodilatation in the muscle itself (22). However, muscular pain occurs in most cases some time after exercise, and there is no evidence that such a histamine release from mast cells or basophils occurs in the postexercise period.

Recently, we demonstrated that in mice brief electrical stimulation of skeletal muscles and prolonged walking both induce within the active muscles a prolonged elevation of the activity of histidine decarboxylase (HDC), the enzyme that forms histamine (11, 29). The histamine newly formed by the induced HDC is released without being stored and has been suggested to play roles in the regulation of the microcirculation (24, 25) and in anabolic processes during rapid cell growth (16). We also found that training of mice diminishes the elevation of muscle HDC activity in the quadriceps femoris muscles induced by walking (11). Moreover, an antagonist of H1 receptors has been shown to improve or tend to improve some symptoms in patients suffering from TMD.

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with TMD (29). Having considered these findings, we proposed the hypothesis that the histamine newly formed through the induction of HDC is involved in mediating the muscular fatigue that occurs after exercise and/or during the development of TMD (11, 29).

In the present study, we examined whether psychological stress with or without muscle activity would induce HDC activity in skeletal muscles in mice. In this study, to assess the systemic effect of stress, we made a comparative examination of the levels of HDC activity not only in various skeletal muscles but also in the stomach. Although the relationship has not been firmly established, gastric HDC activity seems to be potentially useful as an index of biochemical responses to stressful stimuli, because gastric HDC activity has been shown in rats to be increased in response to stresses such as water bathing, restraint, or restraint combined with exposure to cold (1, 4, 26). In the present study, we also looked for signs of hemorrhage in the stomach for much the same reason.

MATERIALS AND METHODS

Animals

BALB/c mice were bred at the animal facility of our university and used as a normal strain in the present study. The mice (male) used for the present experiments were 6–7 wk old (23–25 g body wt). They were kept under standard conditions [12:12-h light-dark cycle (0700–1900 and 1900–0700) at a controlled temperature (23 ± 1°C)] for 7–10 days, and given standard food pellets (LabMR Stock; Nihon Nosan, Yokohama, Japan) and water ad libitum. All experiments complied with the Guidelines for Care and Use of Laboratory Animals issued by Tohoku University.

Stress Experiments

At 0900 on the morning of the day of the experiments, all mice were moved to cages with new wood-chip bedding and kept for 5 h without food but with free access to water. Each stress was imposed on mice for 4 h from 1400 to 1800 at room temperature (23 ± 1°C) as will be described. In each experiment, control mice were kept in the cages without food or water for the same 4-h period.

Water bathing. Water (25°C) was poured into a colorless transparent plastic beaker (diameter 9 cm, height 12 cm) to a depth of 4 cm. Wire netting (5-mm mesh) was placed on the bottom of the beaker to provide a secure footing. Each mouse was put into one of these beakers and kept there for 4 h at room temperature (23 ± 1°C). Water bathing increases when walking is prolonged for >6 h, when experiments are carried out at a higher room temperature (26–28°C), or when the animals used are aged with a heavy body weight. Whenever we observed falling or signs of distress (e.g., vocalization, unsuccessful attempts to walk at the required speed), we immediately removed the mouse from the cage and excluded it from the experiments.

Exposure to cold. Four mice were put into an aluminum cage (width 16 cm, length 30 cm, height 11 cm) with new wood-chip bedding and kept at 7°C for 4 h without food or water.

RestRAINT. The device we used to prevent mice from carrying on their usual activities is shown in Fig. 1. Each mouse was put into a gray plastic cylinder (2.5 cm internal diameter, 10 cm length), the front end of which had been partially closed with a colorless transparent plastic strip (7 cm length, 1.5 cm width, and 1.0 mm thickness). This strip was inserted across the cylinder through two slots near the end (see Fig. 1a). It should be noted that two apertures (about 5 mm maximum width) remain in the front end of the cylinder when the strip is in place (above and below the strip). After each mouse had been placed in the cylinder from the back end, the back end was closed with tape. Interestingly, although the mouse can move back and forth in the cylinder, it

![Fig. 1. The device (a) for restraint with or without mastication. Plastic strips (b) before (left) and after (right) mastication. Note the small depression on the upper edge of the plastic strip before mastication; each mouse started biting at this depression.](http://ajpregu.physiology.org/)
usually bites the plastic strip throughout the whole experimental period (4 h; Fig. 1b). However, if the tail of the mouse is fastened to the back end of the cylinder by tape, the mouse cannot reach the strip and therefore cannot bite it. Using these two methods, we could examine the effect of restraint with or without mastication on HDC activity in the masseter muscle. In this study, these methods are called “restraint with mastication” [R(+)-M] and “restraint without mastication” [R(−)-M], respectively. These experiments were also carried out at room temperature (23 ± 1°C).

Assessment of Masseter Muscle Activity

As described previously, a mouse placed in a narrow cylinder usually bites the plastic strip inserted across the cylinder throughout the whole experimental period. In so doing, it chews away part of the strip (Fig. 1). Therefore, to make a rough assessment of the amount of movement or activity in the masseter muscle (i.e., mastication), we weighed the plastic strips before and after R(+)-M.

Assay of HDC Activity

For the assay of HDC activity mice were decapitated, and the masseter, quadriceps femoris, and pectoralis superficialis muscles were removed and stored at −80°C until assayed. The whole stomach was removed and opened with scissors, washed in ice-cold saline in a glass tube, blotted on a filter paper, and stored at −80°C. HDC activity was assayed with our previously published method (8) with a slight modification (9, 10). Briefly, each tissue sample (<250 mg) was put into a cooled Teflon tube with phosphorylated cellulose (50–100 mg) and 2.5 ml of ice-cold 0.02 M phosphate buffer (pH 6.2) containing pyridoxal 5′-phosphate (20 μM) and dithiotreitol (200 μM) and then homogenized with an Ultra Turrax homogenizer (Janke and Kunkel). The supernatant obtained after centrifugation of the homogenate (10,000 g at 15 min at 4°C) was used as the enzyme solution. The histamine in the tissues was bound to the phosphorylated cellulose and was removed almost completely from the enzyme solution by centrifugation. Reaction mixture (1 ml) containing the enzyme solution was incubated at 37°C for 14 h with histidine (1 mM). After the enzyme reaction had been terminated by adding 2 ml of 0.5 M HClO₄, the histamine formed during the incubation was separated by chromatography on a small phosphorylated cellulose column and quantified fluorometrically (8). HDC activity was expressed as nanomoles of histamine formed during a 1-h period of incubation by the enzyme contained in 1 g (wet weight) of each tissue (nmol·h⁻¹·g⁻¹).

Observation of Gastric Injury

Methods do exist for quantifying gastric lesions in rats, including measurement of the necrotic area after fixation in Formalin solution. However, we could not use this method, because Formalin denatures enzyme proteins and because we needed to freeze the stomach rapidly after its removal for the assay of HDC activity. Therefore, we counted the number of red spots on the gastric mucosa (4) through a magnifying glass when each stomach was put into a glass tube to be washed with cold saline (see Assay of HDC Activity). Any hemorrhagic gastric lesion present was quantified as follows: none, 0 red spots; slight, 1–3 red spots; medium, 4–6 red spots; and strong, >7 red spots.

Data Analysis

Experimental values are given as means ± SD. The statistical significance of the difference between two means was evaluated with Student’s unpaired t-test, and P < 0.05 was considered significant.

RESULTS

Water Bathing and Prolonged Walking

Under the conditions we used for water bathing, each mouse remained for 4 h in an almost stationary standing posture with forefeet on the side of the beaker and the hindfeet on the wire netting at the bottom. On the other hand, the mice in the revolving cage were forced to walk, cling, and/or hang by the forefeet and hindfeet from the rods forming the wall of the cage without rest. Although the prolonged walking induced a marked elevation of HDC activity in all the skeletal muscles tested, the water bathing did not (Fig. 2). Water bathing and prolonged walking each produced an elevation of HDC activity in the stomach. In addition, these stresses resulted in strong hemorrhagic lesions in the stomach of all four mice in each group. It should be noted that the stomach contains a considerable HDC activity even without these stresses (Fig. 2).

Cold Stress and Restraint

Throughout the period of exposure to cold (4 h), the mice remained huddled together with only occasional movements. Nevertheless, HDC activity was enhanced or tended to be enhanced in all skeletal muscles tested as well as in the stomach (Fig. 3), the elevation in the masseter muscle being very clear. As mentioned in MATERIALS AND METHODS, in the experiment involving restraint stress with mastication, each mouse started to bite the plastic strip soon after being shut in the narrow cylinder (Fig. 1b), and this behavior continued almost all the time the experiment lasted. In this experiment, the mouse can move back and forth in the cylinder and can move his forelimbs so as to grip the plastic strip (Fig. 1b). HDC activity in all the skeletal muscles tested, the water bathing did not (Fig. 2).
muscles tested increased in these mice, and the increased levels tended to be greater than those induced by cold stress, although in the stomach the elevation of HDC activity was similar in magnitude to that induced by cold stress. During cold stress, the incidence of hemorrhage in the stomach was one out of four mice (slight), but there was no such hemorrhage in the R(+)M group.

**Effect of Mastication on HDC Activity in Restrained Mice**

As mentioned in MATERIALS AND METHODS when the tail of the mouse was fastened to the back end of the cylinder by tape, the mouse could not bite the plastic strip. Therefore, by comparing data from the two restraint experiments, we could assess the effect of mastication on HDC activity in the masseter muscle. As shown in Fig. 4, HDC activity did not increase at all in the masseter muscle of the R(+)M mice (i.e., without mastication). It also should be noted that the elevation of HDC activity was slight in the superficial pectoralis muscle of the R(+)M mice, although HDC activities in the quadriceps femoris muscle and stomach increased to levels similar to those seen in the R(-)M mice. In this experiment, a slight hemorrhage in the stomach was observed in one mouse in the R(+)M group.

**Relationship Between Mastication and HDC Activity**

As mentioned previously in the R(+)M experiment, the mouse chews the plastic strips and thus the weight of the strip decreases (Fig. 5). In fact, every mouse bit the plastic strip during the whole experimental time (6 h), and HDC activity in the masseter muscle increased with the mastication time (Fig. 5).

**DISCUSSION**

In the present study we found that different types of stress or exercise produce different effects on HDC activity in skeletal muscles in mice, even though mice all showed an elevated HDC activity in the stomach. Prolonged walking (which includes clinging and/or hanging by forefeet and hindfeet), restraint (with or without mastication), exposure to cold, and water bathing all elevated HDC activity in the stomach. In addition, prolonged walking and water bathing produced a high incidence of strong hemorrhage in the stomach. Although both the incidence and severity were lower, hemorrhage also was seen in response to cold stress and R(+)M. The HDC activity induced in the stomach was highest following prolonged walking, whereas the responses induced by the other stresses were weaker and similar to each other. To judge from these results, it seems unlikely that the level of HDC activity in the...
During water bathing the mice made no apparent movements, and there was no detectable elevation of HDC activity in any of the muscles tested. Although each mouse remained standing throughout the experimental period, the buoyancy effect presumably reduced the weight burden imposed on the hindlimbs. On the other hand, during the cold stress small movements were made. Despite the small size of these movements, there was a significant elevation of HDC activity in the masseter muscle and quadriceps femoris muscle and there was a tendency toward an increase in the superficial pectoralis muscle. Possibly, this may have been related to shivering, which was indeed observed in these mice. During R(+)M, the mouse could move back and forth, although with some difficulty. The HDC activities induced in these mice were higher or showed a tendency to be higher than those induced in the mice exposed to cold. These results led us to think that there might be an intimate relationship between the level of HDC activity and the amount of muscle activity or muscle tension.

In the present study we found that throughout R(+)M, each and every mouse, excitedly or eagerly and almost without respite, continued to bite, grip, or pull the strip; this behavior may represent an attempt to escape from the restraint. In these mice, the HDC elevation in the masseter muscle was much higher than in control mice. In contrast, in mice restrained without mastication, such an increase in HDC activity was not detected in the masseter muscle. The elevation of HDC activity in the superficial pectoralis muscle was also higher in R(+)M mice than in R(−)M mice. As shown in Fig. 5, HDC activity in the masseter muscle increased in parallel with the cumulative amount of masticatory activity (as indicated by the weight of the plastic strips). These results strongly support the idea that HDC activity levels induced in skeletal muscles during stress with exercise mainly reflect the strength of the muscle activity or tension and/or the amount of time for which the contractions occur.

In our R(+)M experiment the mice used their masseter muscles voluntarily, i.e., no artificial stimulation was imposed on the muscle. We believe that this experimental system provides us with a singularly useful method for examining the relationship among stress, muscle activity, and the biochemical events accompanying muscle fatigue. We are now using this method to examine how drugs acting on the central nervous system (including psychotropic agents) might affect HDC activity in the masseter muscle and its masticatory behavior.

Interestingly, prolonged walking (and/or hanging by the feet) induced the highest level of HDC activity not only in the quadriceps femoris and superficial pectoralis muscles but also in the masseter muscle (Figs. 2 and 3). The muscular activities of the quadriceps femoris and pectoralis superficial muscles in this experiment were unequivocally the most vigorous among the experiments in the present study. As shown in our previous studies (11, 29), prolonged walking results in an elevation of HDC activity even in the liver, lung, spleen, and bone marrow. A number of cytokines have been suggested as causative factors in the muscular injury that can be induced by exercise (5). We found that small doses of interleukin-1 (IL-1) can elevate HDC activity in all the above tissues (11, 29), and we detected IL-1-like substances in muscle tissues by means of an immunostaining technique (11). This being the case, the elevation of HDC activity in the masseter muscle during prolonged walking might have been induced by cytokines, including IL-1. It is also likely that a degree of muscle tension in the masseter muscle during the prolonged walking (from clenching the teeth, although we could not detect it by eye) might also be involved in the induction of HDC activity.

As mentioned previously, water bathing produced a stomach hemorrhage in all the mice, indicating that this is a potent stress for the mouse. Thus this experiment presumably involved potent psychological or emotional effects. Nevertheless, there was no detectable elevation of HDC activity in any of the muscles tested, including the masseter muscle. On this basis, it seems likely that psychological stress itself does not necessarily induce an elevation of HDC activity in skeletal muscles.

In conclusion, our results suggest that 1) elevation of HDC activity in the stomach may be an index of responses to various types of stress, 2) the elevation of HDC activity in skeletal muscles may be partly or wholly induced by muscle activity and/or tension, and 3) psychological stress itself does not necessarily induce an elevation of HDC activity in skeletal muscles.

**Perspectives**

Our findings in the present study and those reported previously (11, 29) are consistent with our hypothesis that an elevation of HDC activity is involved in inducing fatigue in skeletal muscles. The newly formed histamine in skeletal muscles [possibly generated within vascular endothelial cells as suggested previously (11)] may contribute to the production of muscle pain. Moreover, the dual effects of histamine on the blood vessels [a constrictor effect on the larger vessels and a dilator effect on the finer vessels (12)] might contribute to the ensuing stiffness. Direct electrical stimulation induces an elevation of HDC activity in skeletal muscles even in mast cell-deficient mice (11, 29), and we tentatively suggest that the histamine newly formed as a result of an elevation of HDC activity should be referred to as “neo-histamine” to distinguish it from mast cell histamine. On the basis of our findings, we speculate that the induction of HDC activity in response to muscle activity could be considered part of the animal’s self-defense repertoire. The neo-histamine, by enhancing capillary permeability and causing arteriolar vasoconstriction, may contribute to increases in the supply of O₂.
and nutrients and the removal of CO₂ and other waste products, and may thus aid the recovery from fatigue. It may also stimulate sensory nerve endings, producing pain and so deterring the individual from engaging in further exercise.

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