

Effects of restraint stress on CRF₂ receptor expression in enteric neurons in the rat stomach.

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ABSTRACT

Stress has been found to interrupt gastrointestinal function. Corticotropin releasing factor (CRF) has been associated with stress-evoked acceleration of gastrointestinal functional changes through both central and peripheral mechanisms. The purpose of the present study was to test a hypothesis that stress elevates CRF₂ receptor expression in the rat stomach.

Male adult *Sprague Dawley* rats were placed under restraint stress for 1 hr. Controls were allowed to move freely in their cages without restraint. Animals were euthanized at varying time periods from immediately after the 1 hr stress period to 8 hours later. Segments of the stomach were removed. Whole-mount myenteric plexus preparations were used for immunohistochemical staining for CRF₂ receptors.

It was found that there was no statistically significant change in CRF₂ receptor expression immediately after the 1 hr stress period. At 4 and 8 hours after the stress period there was a significant increase in receptor expression. This increase in CRF₂ receptor expression in the rat stomach may contribute to the slowing of gastric motility.

INTRODUCTION

Stress can profoundly influence gut function. Physical and psychological stress inhibits gastric motility and emptying, which makes one feel full and bloated after eating. Corticotropin releasing factor (CRF) has been implicated in stress-evoked gastrointestinal functional changes through mechanisms in the brain and in the gut itself. Once CRF is released during stress, it binds to the CRF₂ receptors in the brain and the stomach and causes an inhibition on gastric motility and emptying (Martinez et al., 1998; 2002). It is known that stress elevates CRF mRNA in the brain regions involved in regulation of gastric function (Laurach et al., 2009), and we would like to test if stress causes a similar change in the stomach. The goal of the current research project is to learn more about how stress affects the function of the stomach. Understanding this process will provide insight into how to control the negative effects caused by stress.

METHODS

Restraint stress animal model

There are many protocols for stress induction in animal models, including swimming, exercise, foot-shock, maze, water avoidance, and wrap restraint stress. We plan to use an acute wrap restraint stress model because it has been shown to mimic psychological stress in humans. Male adult *Sprague Dawley* rats will be housed in the UW-L animal facility at 22°C with a 12 h light/dark cycle. Food and tap water are available *ad libitum*. We will handle each animal daily for one week before exposed to stress to allow the rats become familiar with us. Rats will be anesthetized lightly with isoflurane, and their shoulders, upper forelimbs and thoracic trunks will be wrapped in cloth tape to restrict, but not prevent, their movements (Williams et al., 1988). The animals will recover from anesthesia within 2 to 5 min and immediately move around in the cages. The mobility of their forelimbs is restricted, which prevents them from grooming the face, the upper head and the neck. Control animals will be anesthetized with isoflurane but will not be wrapped. After recovering from anesthesia, control rats will be allowed to move freely in their cages. The experimental procedures will be performed at the same time of the day, between 9:00-10:00 AM to minimize the effect of circadian rhythm.

Tissue harvest

Immediately after the 1 h stress/or control period, the rats will be euthanized by CO₂ inhalation. The stomach will be removed, washed with ice-cold Krebs solution, and divided into two halves by cutting along the greater and

lesser curvatures. The stomach will be fixed in Zamboni's fixative and used for immunohistochemical staining for CRF₂ receptors.

Immunohistochemical staining of CRF₂ receptors

The effect of stress on CRF₂ receptors expression in the enteric nervous system of the stomach will be studied with immunohistochemical staining. Whole-mount preparations of the myenteric plexus will be dissected from the fixed stomach tissue. The ganglia that house CRF₂ receptors can be seen in this layer. The preparations will be incubated in a solution containing anti-CRF antibody or anti-CRF₂ antibody for 24-48 hours. After that, the preparations will be washed in phosphate-buffered saline (PBS) and then incubated in appropriate fluorescence-tagged secondary antibody. The preparations will be rinsed thoroughly, mounted on a glass slide, coverslipped with mounting medium and examined under a Nikon Eclipse E600 fluorescence microscope. When examining the slides, 30 randomly chosen ganglia throughout the tissue will be analyzed for the number of neurons showing CRF₂ immunoreactivity. The average number of CRF₂ neurons per ganglion will be calculated as well as the standard error. A T-test will be used to determine if the difference between the control and stress-restrained rats is statistically significant.

RESULTS

The number of CRF₂ receptors increased after an hour of restrained stress, but it was not statistically significant (see Figure 1, below). Four and eight hours after the hour of restrained stress indicated a significant increase in the amount of CRF₂ receptor expression.

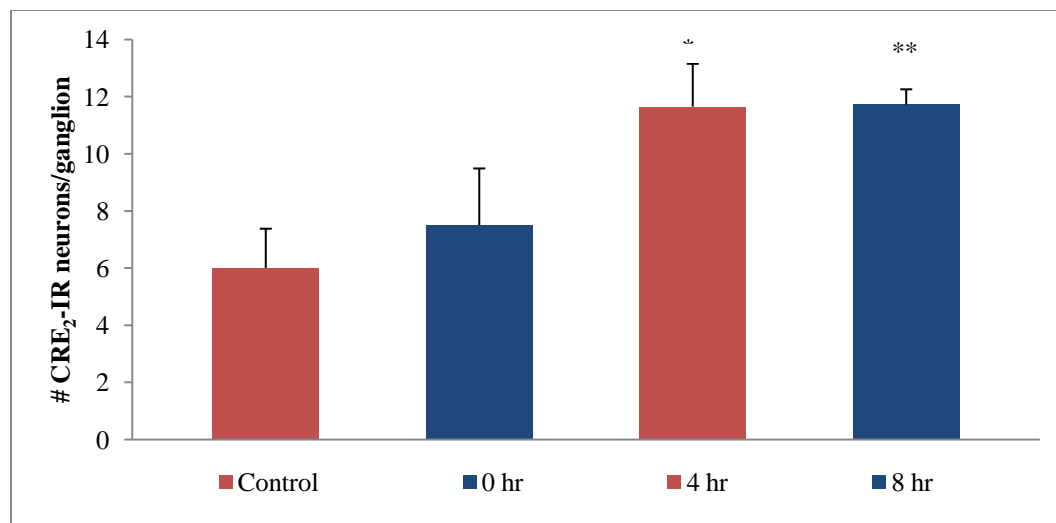


Figure 1. Quantitative data showing the effects of restraint stress on the number of CRF₂ receptor immunoreactivity in the myenteric plexus of the stomach. * P < 0.05; ** P < 0.005.

DISCUSSION

Restraint stress elevates CRF₂ receptor expression in the myenteric plexus of the rat stomach four hours after the hour of restrained stress. According to the data, it takes four hours for the number of CRF₂ receptors to statistically increase. This increase in CRF₂ receptor expression in the rat stomach may contribute to the slowing of gastric motility. Further research could be performed to determine how long the CRF₂ receptor expression is increased.

This research was reported at National Conference of Undergraduate Research and at the University of Wisconsin-La Crosse Celebration of Research and Creativity.

LITERATURE CITED

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