Role of Thyrotropin-Releasing Hormone in Stress Ulcer Formation in the Rat

NICOLA BASSO, MD, MAURIZIO BAGARANI, MD, A. EUGENE PEKARY, MD, ALFREDO GENCO, MD, and ALBERTO MATERIA, MD

The role of the hypothalamic peptide thyrotropin-releasing hormone in stress ulcer formation was investigated. In experiment 1, TRH was peripherally administered (10 μg/kg) to rats subjected to cold-restraint stress and compared to an inactive peptide; in experiment 2, TRH was administered intracerebroventricularly (0.02, 0.1, and 0.5 μg/kg) to rats with no adjunctive experimental stress; in experiment 3, TRH antiserum was given intracerebroventricularly to rats subjected to stress and compared to normal rabbit serum. When TRH was administered subcutaneously in rats subjected to stress, it significantly aggravated ulcer formation, and this effect was inhibited by atropine and vagotomy. When administered intracerebroventricularly, TRH alone induced, in a dose-dependent fashion, the formation of gastric ulcers. TRH antiserum infused intracerebroventricularly inhibited ulcer formation induced by cold-restraint stress. In conclusion, TRH seems to play a role in stress ulcer formation, possibly by a cholinergic mediated mechanism.

KEY WORDS: thyrotropin-releasing hormone; hypothalamus; stress ulcer.

Physiological and pathophysiological observations have established a strong interplay between the central nervous system and the gut. In particular, the vagus and several neuropeptides have been shown to exert a major physiological control on gastric secretory and motor functions (1-3). It is also well documented that acute gastric mucosal lesions may result from severe head injuries and following neurosurgical procedures (4-6). However, although several studies have tried to correlate these stress-induced changes in the central nervous system with gastric effects (7-9), none was able to identify a common mediator or pathway.

Experimentally, stimulation or destruction of different hypothalamic regions has been successfully correlated with gastric ulcer formation, thus suggesting a prominent role of mediation by the hypothalamus (4, 10). The mechanisms by which the hypothalamus may play a role in stress ulcer formation are largely unknown. Several studies have recently shown that hypothalamic peptides exert a number of significant effects on the gastrointestinal tract (1, 11). In particular, thyrotropin-releasing hormone (TRH), which has been most extensively studied, has been reported to stimulate gastric acid secretion when administered intracerebroventricularly in the rat (12). Indirect evidence suggests that TRH and corticotropin releasing factor (CRF) release is augmented by stress (1), but whether these
peptides are involved in the genesis of stress ulceration is unknown.

The purpose of this study was threefold: (1) to assess whether peripherally administered TRH could aggravate stress ulcer formation, (2) to determine whether TRH administered intracerebroventricularly could induce the formation of gastric mucosal lesions in the absence of experimental stress, and (3) to determine whether pretreatment with TRH antiserum could antagonize stress ulcer formation.

MATERIAL AND METHODS

Male Sprague-Dawley rats, weighing 220–250 g, were utilized. Prior to study, the animals were housed in large cages in a room maintained at constant temperature with 12:12 hr light–dark cycles. The rats were fed a standard diet of commercial rat chow and were conditioned to the room environment for at least 15 days prior to inclusion in the studies. All rats were deprived of food but not of water for 24 hr prior to the beginning of the experiments and were housed in individual cages with wide mesh wire bottoms to prevent coprophagia. All experiments were performed between 8 AM and 2 PM.

In experiment 1, the rats were administered saline, 1 ml subcutaneously, (N = 6) or TRH, 10 ~g/kg subcutaneously (N = 6), or anorexigenic tripeptides (AT), 100 ~g/kg subcutaneously; AT is a peptide identical to TRH, except for one substituted amino acid, and functioned as inactive control.

After 30 min all rats were stressed by a modification of the methods of Brodie and Hanson (13) and Brodie and Valitsky (14). Using this technique, the animals were placed in specially designed cylindrical steel restraint cages and placed in a cold room (4 °C) for 4 hr. At the end of the treatment period, all rats were sacrificed by ether overdose. After sacrifice, the abdomen was opened by a midline incision, the stomach was removed and opened along the greater curvature, the mucosa rinsed with tap water, and the severity of the gastric erosions was evaluated in a blinded fashion using a dissecting microscope. Only gastric mucosal lesions occurring in the glandular part of the mucosa were considered. The length and width of each lesion were measured to the nearest 0.2 mm, and the area of ulceration was calculated as an ellipse as described by Kauffman and Grossman (15). An arbitrary ulcer index was calculated based on the raw sum of the areas of each ulceration in a single rat. The average ulcer index for a group of animals was calculated as the mean of the ulcer indexes of each rat ± the standard error of the mean (SEM).

The experiments were repeated with the same design after a 30-min pretreatment with atropine (1 mg/kg subcutaneously) (N = 6). Twelve additional rats were also studied with the same protocol 10 days after a surgical truncal vagotomy or a sham operation. At the end of these experiments, the severity of the stress-induced gastric mucosal lesions was similarly evaluated.

In experiments 2 and 3 the rats were anesthetized with ether and the head positioned in a specially designed stereotactic frame (16). A steel cannula (15 x 1.2 mm) was positioned into the left lateral ventricle according to a previously described technique (17). The cannula was connected by plastic tubing to a microosmotic pump (Alzet 1702, Alza Corp., Palo Alto, CA) with a total capacity of 166 ± 5.0 ~g. Prior to insertion, the osmotic pump was pretreated with 0.9% NaCl solution for 24 hr. Once the pump was connected to the intraventricular cannula, it was positioned in a subcutaneous pouch in the dorsal region of the rat. In experiment 2 (N = 6) the solutions, which were infused at a constant rate of 0.55 ~g/hr for 4 hr, were TRH at the doses of 0.02, 0.1, 0.5 ~g/kg/hr and 0.9% NaCl solution. In experiment 3 (N = 8) the rats were pretreated by intracerebroventricular infusion of control serum or TRH antiserum (18) for 24 hr and then subjected to cold-restraint stress as previously described. The TRH antiserum had negligible in vitro cross-reactivity with all other hypothalamic peptides including met-enkephalin, leu-enkephalin, and beta-endorphin. In all rats of experiments 2 and 3 the average ulcer index was evaluated as in experiment 1.

The correct position of each cannula into the lateral ventricle was controlled by anatomical cerebral dissection at the end of each experiment.

RESULTS

Experiment 1. The mean ulcer index of rats subjected to cold-restraint stress with or without TRH pretreatment is shown in Figure 1. Subcutaneously administered TRH significantly aggravated stress-ulcer formation (P < 0.05) whereas AT had

Fig 1. Mean ulcer index in rats subjected to cold-restraint stress after pretreatment with TRH (10 ~g/kg subcutaneously) or AT (100 ~g/kg subcutaneously) (N = 6).