Central action of adrenomedullin to prevent ethanol-induced gastric injury through vagal pathways in rats

HIROSHI KANEKO,1 TERUNORI MITSUMA,1 HIROFUMI NAGAI,1 SHOZABURO MORI,1 TAKASHI IYO,1 KAZUO KUSUGAMI,2 AND YVETTE TACHE3

1Fourth Department of Internal Medicine, Aichi Medical University, Aichi 480-1195; 2First Department of Internal Medicine, Nagoya University School of Medicine, Nagoya 466-8560, Japan; and 3Center for Ulcer Research and Education/Digestive Disease Research Center, West Los Angeles Veterans Affairs Medical Center, Department of Medicine and Brain Research Institute, University of California, Los Angeles, California 90073

Kaneko, Hiroshi, Terunori Mitsuma, Hirofumi Nagai, Shozaburo Mori, Takashi Iyo, Kazuo Kusugami, and Yvette Taché. Central action of adrenomedullin to prevent ethanol-induced gastric injury through vagal pathways in rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1783–R1788, 1998.—Adrenomedullin (AM), belongs to the calcitonin gene-related peptide (CGRP) family and interacts with AM and CGRP1 receptors. Specific AM receptors and immunoreactivity are present in the rat brain. The effect of intracisternal injection of rat AM on ethanol-induced gastric lesions was studied in conscious Wistar rats. The peptide was injected intracisternally or intravenously under short anesthesia 20 min before intragastric injection of 70% ethanol. Corpus lesions were determined 1 h after ethanol. Intracisternal AM (75, 150, and 300 pmol) dose-dependently inhibited ethanol-induced gastric lesions by 40–72% and rat α-CGRP (150 pmol ic) by 76%. Intravenous AM (300 pmol) had no effect. The CGRP1 receptor antagonist CGRP-(8—37) (9.6–19.2 nmol ic) dose-dependently inhibited the protective effect of intracisternal α-CGRP but not that of AM. Subdiaphragmatic vagotomy and peripheral injection of atropine, indomethacin, or Nω-nitro-L-arginine methyl ester (L-NAME) prevented AM protective action. L-Arginine but not D-arginine blocked L-NAME action. These data suggest that both AM and CGRP act in the brain to prevent ethanol-induced gastric lesions through interaction with their specific receptors. AM action may involve vagal cholinergic-dependent modulation of prostaglandins and nitric oxide protective mechanisms.

calcitonin gene-related peptide antagonist; nitric oxide; prostaglandins; atropine; vagus

ADRENOMEDULLIN (AM) is a 52-amino acid peptide recently isolated from a human pheochromocytoma by Kitamura et al. (14). Subsequent cDNA cloning of the human, rat, and porcine AM showed high homology across species (14). Rat AM is similar but distinct from human AM, with two amino acid deletions and six substitutions (34). The peptide bears homology to calcitonin gene-related peptide (CGRP), calcitonin, and amylin, which have an NH2-terminal ring structure of six to seven amino acids involving a disulfide bridge and an amidated COOH-terminal end (14, 34). In the COOH-terminal portion (amino acid residues 16–52), AM shares an ~27% homology with CGRP (34). Recently, the cDNA for the rat and human AM and CGRP1 receptors have been cloned (1, 6, 10, 11). Comparison of the peptide sequence of AM and CGRP and seven-transmembrane domain receptors indicates a 30% identity between them (11). Characterization of these receptors in transfected cells as well as in binding studies on various membranes indicate that AM displays a highly specific recognition at the AM receptor over CGRP but also cross-reacts with the CGRP1 receptor (1, 10, 11, 20, 37). In common with CGRP, AM injected intravenously was first reported to elicit a strong and long-lasting hypotensive response in rats (14, 22). Thereafter, there has been considerable interest in establishing the in vivo and in vitro specific actions of the peptide in the cardiovascular system (14, 21). In particular, several studies established that AM vasodilatory properties in various vascular bed preparations are mediated by selective interaction with the AM receptor and/or cross talk with the CGRP1 receptor, as assessed by the use of the CGRP receptor antagonist CGRP-(8—37) (7, 14, 21).

Growing evidence also indicates that AM acts in the central nervous system to influence feeding behavior, sympathetic outflow, and cardiovascular and gastric motor function in rats (16, 19, 23, 29, 30, 32). A hindbrain site of action for intracisternal injection of AM-induced increases in blood pressure and heart rate was recently located in the area postrema, where AM activates a population of neurons (2, 3). The localization of AM immunoreactivity and binding sites in the brain stem (9, 20, 32) suggests a possible role of medullary AM. We previously reported that α-CGRP injected intracisternally inhibits experimental models of gastric erosions [cold restraint, intracisternal injection of thyrotropin-releasing hormone (TRH), ethanol] induced or modulated by vagal-dependent mechanisms (25, 27). Whether intracisternal injection of AM influences the resistance of the gastric mucosa to injury through vagal-dependent pathways has not yet been explored. The present study was designed 1) to examine whether AM injected intracisternally acts in the brain to reduce ethanol-induced gastric lesions, 2) to establish whether the protective effects of α-CGRP and AM injected intracisternally are sensitive to blockade by intracisternal injection of the CGRP antagonist CGRP-(8—37), and 3) to define the neurohumoral pathways involved in AM action, in particular the role of vagal cholinergic pathways previously shown to increase the resistance of the mucosa to ethanol-induced gastric injury through prostaglandins and NO-dependent mechanisms (28).
METHODS

Animals. Male Wistar rats (specific pathogen free, 200–300 g; Japan Shizuoka Laboratory Center, Hamamatsu, J apan) were maintained with a standard diet of laboratory chow and tap water ad libitum under conditions of controlled temperature (23 ± 2°C), humidity (60 ± 5%), and illumination (12-h light cycle starting 7:30 AM) for at least 15 days before the experiments. Rats were deprived of food but not water for 24 h before the beginning of the experiments. Experimental protocols were approved by the animal care and use committee of Aichi Medical University.

Chemicals and treatments. The following substances were used: rat AM (1–50; no. 4281-s), rat α-CGRP (no. 4163-s), human CGRP-(8–37) (no. 4232-v; Peptide Institute, Osaka, J apan), BSA, atropine sulfate, indomethacin, N0-nitro-L-arginine methyl ester (L-NAME), L-arginine, D-arginine, ketamine hydrochloride (Sigma, St. Louis, MO), ether, and ethanol (Wako Chemical, Osaka, J apan). Peptides were freshly dissolved in saline containing 0.1% BSA immediately before the experiment. Atropine, L-NAME, L-arginine, and D-arginine were dissolved in saline. Indomethacin was dissolved in 1.0% NaHCO3 solution. Ethanol was diluted in distilled water. Seventy percent ethanol (5 ml/kg) was given intragastrically by an oral gavage using a stainless steel cannula. Atropine was injected subcutaneously and indomethacin was injected intraperitoneally in 1.0 ml/kg in lightly restrained conscious rats. Intracisternal injections into the cisterna magna and intravenous injections into the jugular vein were performed in volumes of 10 µl/rat and 1.0 ml/kg, respectively, in rats under brief ether anesthesia. Subdiaphragmatic vagotomy or sham operation was performed in fasted rats under ketamine (100 mg/kg ip) anesthesia 16 h before the experiment.

Experimental protocols. In the first experiment, rats under ether anesthesia were injected with either rat AM (30, 75, 150, or 300 pmol ic or 300 pmol iv) or vehicle (intracisternal or intravenous saline containing 0.1% BSA). In the second experiment, the CGRP receptor antagonist human CGRP-(8–37) (9.6, 19.2, or 28.8 nmol/10 µl) or vehicle was injected intracisternally at 9.6 nmol (30 µg) immediately followed by intracisternal injection with α-CGRP (150 pmol), AM (150 pmol), or vehicle (10 µl). In the third experiment, rats with sham operation or subdiaphragmatic vagotomy (−16 h) were injected intracisternally with either AM (150 pmol) or vehicle (10 µl). In other groups, atropine (0.15 mg/kg sc, −30 min), indomethacin (5 mg/kg ip, −60 min), or L-NAME (3 mg/kg iv, −1 min) was administered before the intracisternal injection of AM (150 pmol) or vehicle (10 µl). In an additional study, L-arginine or D-arginine (300 mg/kg iv) was injected immediately before L-NAME (3 mg/kg iv). The regimen of indomethacin, L-NAME, and L-arginine or D-arginine administration was based on previous studies that showed the complete blockade of the vagal-dependent gastric cytoprotection induced by intracisternal injection of the TRH analog RX-77368 under otherwise similar conditions (13, 35). In all treated groups, 70% ethanol was administered intragastrically 20 min after intracisternal injection of peptide or vehicle. Conscious rats were euthanized by decapitation under ether anesthesia 1 h after ethanol administration.

Measurement of gastric mucosal lesions. After ligation of the pylorus and the esophagus at the gastric junction, stomachs were removed, inflated with 10 ml of 0.5% Formalin solution, and lightly fixed by immersion in 0.5% Formalin solution for 10 min. The gastric mucosa was exposed by cutting the stomach along the greater curvature, washed with saline-wetted gauzes, laid on a flat surface, and photographed. The outline of the gastric corpus and the localization of the lesions characterized by dark bands on the pictures were traced onto a transparent film using a felt-tip marker by a trained person unaware of the treatment regimen. The percentage of the gastric corpus containing lesions was determined by a computed image analyzer device (Macintosh LC 630; Apple J apan, Tokyo, J apan; application with National Institutes of Health Image 1.55 and Adobe Photoshop 3.0) equipped with an imaging scanner (GT-8000; Epson, Tokyo, J apan).

Statistics. Results are expressed as means ± SE. Multiple group comparisons were performed by a one-way ANOVA followed by a Dunnett’s contrast. Comparisons between two groups were performed by Student’s t-test. A probability level of P < 0.05 was considered significant.

RESULTS

Oral administration of 70% ethanol (5 ml/kg) produced macroscopic gastric lesions visualized as long dark red bands linearly oriented along a cranial to caudal axis and primarily confined to the proximal corpus mucosa. Gastric mucosal lesions covered 10.4 ± 2.0 and 12.1 ± 1.5% of the corpus mucosa in rats injected with vehicle into the cisterna magna or jugular vein, respectively (Fig. 1). Intracisternal injection of AM at 75, 150, and 300 pmol significantly inhibited ethanol-induced lesions to 6.2 ± 0.8, 3.1 ± 0.5, and 2.9 ± 0.4% of the gastric mucosa, respectively, whereas at 30 pmol, intracisternal AM had no effect (11.4 ± 0.9%) compared with the vehicle-treated group (Fig. 1). In contrast to the intracisternal injection of AM (75–150 pmol), which dose-dependently inhibited ethanol-induced gastric injury by 42–70%, the peptide injected intravenously at 300 pmol had no effect (Fig. 1).

Intracisternal injection of α-CGRP at 150 pmol inhibited ethanol-induced gastric mucosal lesions by 76% (Fig. 2). α-CGRP-induced gastric protection was partly prevented by the CGRP receptor antagonist CGRP-(8–37) injected intracisternally at 9.6 nmol (30 µg) immediately before α-CGRP and was completely blocked when...
CGRP-(8—37) was injected at 19.2 nmol (60 µg) (Fig. 2). By contrast, CGRP-(8—37) (19.2 nmol ic) did not modify the gastric protective effect of AM (150 pmol) injected into the cisterna magna under the same conditions (Fig. 2). Intracisternal injection of CGRP-(8—37) alone at 9.6 or 19.2 nmol did not influence ethanol-induced gastric lesions, whereas a higher dose (28.8 nmol) had a significant 36% inhibitory effect (Fig. 2).

In sham-operated rats, orogastric administration of 70% ethanol resulted in 18.8 ± 1.4% lesions of the corpus mucosa that were reduced by 74% in rats injected intracisternally with AM (150 pmol) (Fig. 3). Subdiaphragmatic vagotomy completely abolished AM (150 pmol ic)-induced gastric protection (P < 0.01) (Fig. 3). Vagotomy itself did not influence ethanol-induced gastric injury (Fig. 3). Pretreatment with atropine (0.15 mg/kg sc) and indomethacin (5 mg/kg ip) prevented the protective effect of AM (150 pmol ic) on ethanol-induced gastric injury by 82 and 100%, respectively (P < 0.01) (Fig. 4). Neither atropine nor indomethacin influenced ethanol-induced lesions in intracisternal vehicle-injected rats (Fig. 4). L-NAME (3 mg/kg iv) also suppressed the gastric protective effect of intracisternal AM (150 pmol) by 91% (P < 0.01) (Fig. 5). L-Arginine, but not L-arginine, injected intravenously at 300 mg/kg before L-NAME (3 mg/kg iv) restored the cytoprotective action of AM injected intracisternally (Fig. 5).

**DISCUSSION**

AM (75–150 pmol) injected into the cisterna magna dose-dependently inhibited gastric injury induced by orogastric administration of 70% ethanol by 42–70% in conscious, fasted rats. At 150 pmol injected intracisternally, AM induced a maximal protective effect, because 300 pmol resulted in a similar 72% inhibition of ethanol lesions. In contrast to the intracisternal route of administration, AM injected intravenously at two times the maximal effective dose given intracisternally had no effect. These results indicate that the gastric protection induced by AM injected into the cisterna magna is mediated through the central nervous system and does not represent a leakage of the peptide into the periphery (4). Other studies showed that intracerebroventricu-
compared with intravenous vehicle. First, the intracisternal AM-induced gastric protection does not involve an action on AM receptors (1, 10, 11, 20, 37). However, intracisternal AM when tested at a high CGRP antagonist concentration (100 pmol) resulted in a similar 70% protection against 70% ethanol injury at higher doses (0.3–5 nmol) increased blood pressure and abdominal sympathetic activity and decreases food intake (29, 30, 32), whereas similar or lower doses injected intracerebroventricularly were reported to inhibit salt appetite and experimentally induced drinking behavior and, given intracisternally, to delay gastric emptying (16, 19, 23).

AM shares some similar spectrum of activity with CGRP (14, 21, 34). In the present study, intracisternal injection of α-CGRP at an equimolar dose as AM (150 pmol) resulted in a similar 70% protection against 70% ethanol-induced gastric injury in conscious rats. These results are in agreement with our previous report in which α-CGRP injected intracisternally in doses ranging from 8 to 80 pmol prevented 40% ethanol-induced gastric lesions by 76–97%, respectively (25). In addition, α-CGRP injected intravenously had no effect on ethanol-induced gastric lesions (15) as observed for intravenous injection of AM. AM displays a slight (25–30%) sequence homology with CGRP at both the peptide (14) and receptor levels (10, 11). In addition, binding studies in several tissues including rat brain membranes and in transfected cells indicate that AM activates the AM receptor as well as interacts with the CGRP receptor, whereas CGRP is devoid of agonist action on AM receptors (1, 10, 11, 20, 37). However, several sets of evidence indicate that intracisternal AM-induced gastric protection does not involve an interaction with the CGRP receptor. First, the intracisternal injection of CGRP-(8–37) immediately before that of AM did not modify the gastric protective effect of intracisternal AM when tested at a high CGRP antagonist/AM molar ratio (128:1). Second, intracisternal CGRP-(8–37) completely abolished the cytoprotective effect of intracisternal α-CGRP at a similar 128:1 ratio. Such conditions seem to be optimal to assess the CGRP receptor involvement in AM action, because at a higher dose, CGRP-(8–37) injected intracisternally displays an agonist action in agreement with neurobehavioral effects observed after intracerebroventricular injection of CGRP-(8–37) at doses reaching 80 µg (25.6 nmol) (8). In addition, previous studies showed that intracerebroventricular injection of CGRP-(8–37) reversed intracerebroventricular AM-induced inhibition of food intake and increase in blood pressure at CGRP-(8–37)/AM molar ratios of 17:1 and 0.7:1, respectively (29, 30). Taken together, these results suggest that intracisternal injection of α-CGRP and AM to induce gastric cytoprotection against ethanol are mediated by specific interactions with their respective receptors.

Gastric protection elicited by AM injected intracisternally does not seem to be secondary to enhanced emptying of ethanol from the stomach. A recent study using AM injected intracisternally at a similar dose as in the present protocol (150 pmol) inhibited gastric emptying of a liquid meal in conscious rats (16). Therefore, changes in gastric transit cannot account for the gastric protection against ethanol injury under the present experimental conditions. It is also unlikely that the central action of AM is secondary to changes in blood pressure, because the hypertensive response was reported to occur at intracerebroventricular or intracisternal doses in the 300–800 pmol range (29), whereas lower doses (88–176 pmol icv) did not alter mean arterial blood pressure in rats (19). In addition, central injection of CGRP-(8–37) reversed the hypertensive response induced by intracisternal or intracerebroventricular injection of AM (29) while not altering the cytoprotective effect induced by intracisternal AM (present observation).

Previous studies indicate that the peripheral mechanisms through which peptides injected centrally protect the gastric mucosa against ethanol lesions relate to the autonomic nervous system-dependent changes in the release of gastric protective factors (12, 13, 33, 35). The protective role of prostaglandins and endogenous NO against ethanol-induced gastric injury has been well established (17, 33). There is also evidence that gastric prostaglandins and NO release are under a stimulatory vagal cholinergic control (12, 24, 36). In the present study, subdiaphragmatic vagotomy, blockade of muscarinic receptors by peripheral injection of atropine, and gastric prostanoid synthesis by intraperitoneal injection of indomethacin abolished the protective effect of intracisternal AM. The observation that indomethacin blocked the gastric protection induced by intracisternal AM whereas it did not influence that of intracisternal α-CGRP (25) further supports the contention that peptide actions are initiated by different receptors. In addition, L-NAME, injected intravenously at a dose that inhibits NO synthesis from L-arginine (18) completely abolished the cytoprotective effect of AM. The action of L-NAME was reversed in an enantio-
Central adrenomedullin-induced gastric cytoprotection

In a meric, specific manner by L-arginine, a substrate for NO synthase, whereas D-arginine was inactive. In intracisternal vehicle-treated rats, no significant changes in ethanol-induced gastric injuries were observed after atropine, indomethacin, or L-NAME pre-treatment as previously reported in conscious rats (13, 35). Taken together, these results suggest that intracisternal injection of AM induces vagal cholinergic activation of the L-arginine-NO synthase and prostaglandin-dependent protective mechanisms. Other studies showed that intracisternal or intracerebroventricular injection of AM-induced delayed gastric emptying, increased sympathetic outflow, and hypertensive response are mediated by CGRP receptor-dependent activation of sympathetic adrenal pathways (16, 29, 30). The present findings represent the first demonstration of a central action of AM mediated through AM receptors and vagal-dependent cholinergic pathways.

The physiological role of CGRP-related peptides in the regulation of the resistance of the gastric mucosa to injury is still to be established. The CGRP1 receptor antagonist injected intracisternally at a dose that blocked exogenous injection of α-CGRP did not influence the development of gastric lesions induced by 70% ethanol. These results suggest that endogenous CGRP does not exert a tonic modulation of gastric mucosal resistance under these conditions of ethanol injury in conscious rats. The lack of specific AM receptor antagonist precludes the assessment of the role of brain AM. However, the demonstration of specific binding sites for AM in rat brain stem by binding and autoradiography studies (20) along with the presence of AM immunoreactivity in the rat medulla, including the midline raphe and the dorsal vagal complex (9, 32), support a role at these sites. AM injected intracerebroventricularly at low doses (80–160 pmol) similar to those in the present study inhibits salt appetite, and such biological action of AM was established to have physiological significance in the central regulation of sodium homeostasis during hypovolemia, as assessed by intracerebroventricular injection of AM antibody (23). Whether AM may increase the resistance of the gastric mucosa through autonomic modulation of defensive mechanisms under pathophysiological conditions associated with the central release of this peptide (31) remains to be established.

In summary, the present data indicate that intracisternal AM and α-CGRP injected at picomole doses act in the brain to protect the gastric mucosa against ethanol injury in conscious rats. Results obtained with the CGRP1 receptor antagonist CGRP-(8–37) injected intracisternally indicate that the central action of α-CGRP is mediated by an interaction with CGRP receptors, whereas that of AM is unrelated to the cross talk of AM with the CGRP1 receptor. The central action of AM appears to be mediated through modulation of vagal cholinergic-dependent protective mechanisms involving prostaglandins and NO. This represents the first demonstration of a central action of AM mediated through autonomic vagal pathways and unrelated to interaction with central CGRP receptors.

Perspectives

The present results indicate that low doses of AM injected intracisternally act through central AM receptors to elicit a vagal cholinergic activation of prostaglandins and NO-dependent protective mechanisms against ethanol-induced gastric injury in conscious rats. Medullary TRH was previously established to protect the gastric mucosa against ethanol injury through similar vagal cholinergic activation of prostaglandin E2 and NO protective mechanisms (12, 27, 28, 35, 36). Whether AM action is mediated by modulating the release of TRH contained in nerve terminals synapsing on neurons of the dorsal motor nucleus of the vagus (26) needs to be investigated further. In vitro single-unit recording demonstrated a direct excitatory effect of AM on area postrema neurons (2), which are known to project to the caudal dorsal vagal complex (5). Mapping studies will be required to localize the brain stem site(s) at which AM acts to induce vagal cholinergic-dependent gastric cytoprotection.

P. Kirsch is acknowledged for helping in the preparation of the manuscript.

This study was supported by the Grant-in-Aid for Encouragement of Young Scientists 07770402 (H. Kaneko); a Grant-in-Aid for Scientific Research (C) 09670586 (H. Kaneko, T. Mitsuma, and S. Mori) from the Ministry of Education, Science, Sports and Culture, Japan; the National Institute of Mental Health, Grant MH-00663 (Y. Taché); and the National Institute of Diabetes and Digestive and Kidney Diseases, Grant DK-30110 (Y. Taché).

Address for reprint requests: H. Kaneko, Fourth Dept. of Internal Medicine, Aichi Medical Univ., 21 Karimata, Yazako, Nagakute, Aichi-gun 480–1195, Japan.

Received 17 November 1997; accepted in final form 18 February 1998.

REFERENCES
