Capsaicin-Induced Calcitonin Gene-Related Peptide Release from Isolated Rat Stomach Measured with a New Chemiluminescent Enzyme Immunoassay

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ABSTRACT—The peripheral capsaicin-sensitive afferent nerve has been reported to play an important role in gastroprotection and to release a calcitonin gene-related peptide (CGRP). We developed a new chemiluminescent enzyme immunoassay (CLEIA) for CGRP and measured capsaicin-induced CGRP release from the isolated and inverted rat stomach. The basal CGRP release from the stomach was 0.40±0.02 pg/mg wet weight in a 30-min incubation. Capsaicin (1×10⁻⁸-1×10⁻⁵ M) stimulated CGRP release in a concentration-dependent manner. In the stomach from rats with defunctionalization of afferent neurons, the levels of the basal and capsaicin-induced CGRP release were below the limit of detection. On the other hand, the capsaicin-induced CGRP release was not blocked by tetrodotoxin treatment. The gangliosym- pathectomy abolished the increase in the CGRP levels. However, the capsaicin-induced CGRP release was not affected by pretreatment with 6-hydroxydopamine, a neurotoxin that causes a complete degeneration of adrenergic nerve terminals. In conclusion, the CLEIA system may be useful for detecting the released CGRP and studying the activity of capsaicin-sensitive nerves, particularly the CGRP-containing nerves. Our results also confirmed that although the CGRP-containing nerve runs in the sympathetic nerve trunk, the activity of the nerve is not affected by adrenergic nerves, and the capsaicin-induced CGRP release may be attributable to the tetrodotoxin-resistant component.

Keywords: Calcitonin gene-related peptide, Capsaicin, Chemiluminescent enzyme immunoassay

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide, the structure of which has been predicted on the basis of alternative processing of the primary transcript of the rat calcitonin gene (1). CGRP immunoreactivity has been reported in both the central and peripheral nervous systems (2-7). Peptide-containing sensory nerves at the visceral levels not only transmit interoceptive information to the brain, but can also exert a local "effector" function by transmitter release from their peripheral endings (8, 9).

Recently, capsaicin-sensitive nerve has been reported to play an important role in gastroprotection. Recent studies suggested that capsaicin-sensitive afferent neurons are involved in the pathophysiological regulation of gastric functions such as secretion and mucosal blood flow (GMBF) (10) and contribute to the mucosal protective mechanism against noxious stimuli (11-14). The mammalian stomach is densely innervated by capsaicin-sensitive primary afferent neurons containing CGRP (15). The gastroprotective action of afferent nerve stimulation by intragastric capsaicin is most likely brought about by a local release of transmitter substance within the gastric mucosa (12-16). CGRP as well as capsaicin is able to increase gastric mucosal blood flow and to protect against ethanol- and aspirin-induced mucosal injury (17). CGRP would thus seem to be a candidate mediator of both the gastric vasodilator and protective effect of afferent nerve stimulation. The aim of this study was to determine if capsaicin itself is capable of stimulating the release of CGRP to the gastric lumen from the gastric mucosa using isolated whole stomach.

Enzyme immunoassay (EIA) can be defined as an immunological procedure in which the antigen-antibody reaction is monitored by enzyme measurements. Development of substrates to be cleaved by enzymes has advanced from colorimetric to fluorometric substances and then to chemiluminescent substances, with a resultant increase in sensitivity. EIA has become more than simply a non-
isotopic alternative to radioimmunoassay (RIA). We have developed the chemiluminescent enzyme immunoassay (CLEIA) method for CGRP determination using disodium 3-(2”-spirod adamantane)-4-methoxy-4-(3”-phosphoryloxy)phenyl-1,2-dioxetane (AMPPD<sup>TM</sup>) as a substrate for alkaline phosphatase and measured capsaicin-induced CGRP release from the isolated and inverted rat stomach.

MATERIALS AND METHODS

**Animals**

Male Sprague-Dawley rats (Charles River Japan, Inc., Yokohama) weighing 170–350 g were used in all experiments. They were housed in a controlled environment at 22±2°C with alternate cycles of 12 hr light and 12 hr darkness and with food (F-2; Sankyo Labo Service Corporation, Inc., Tokyo) and water ad libitum.

**Drugs**

The following drugs were used: affinity purified Goat anti-Rabbit IgG (H+L) (Jackson Immuno Research Laboratories, Inc., West Grove, PA, USA); rat calcitonin gene-related peptide (CGRP) (Peptide Institute, Osaka); Block Ace® (Yukijirushi Nyugyou, Sapporo); rabbit anti-CGRP serum and biotinyl-CGRP (Peninsula Laboratories, Inc., Belmont, CA, USA); streptoavidine-alkaline phosphatase (Life Technologies, Inc., Gaithersburg, MD, USA); disodium 3-(2”-spirod adamantane)-4-methoxy-4-(3”-phosphoryloxy)phenyl-1,2-dioxetane (AMPPD<sup>TM</sup>) (Tropix, Bedford, MA, USA); tetrodotoxin (TTX) (Wako, Osaka); capsaicin, terbutaline and aminophylline (Sigma, St. Louis, MO, USA); 6-hydroxydopamine (6-OHDA) (Aldrich, Milwaukee, WI, USA).

**CLEIA for CGRP**

Microassay plates were coated with 50 µl per well of affinity purified Goat anti-Rabbit IgG (H+L) at a concentration of 2.5 µg per ml in Tris/HCl buffered saline (TBS; pH 7.5, 50 mM). Then the wells were washed three times with 300 µl washing buffer (50 mM Tris/HCl buffer containing 0.154 M NaCl and 0.05% Triton X-100). The wells were subsequently filled with a Block Ace diluted fourfold in TBS and incubated at least 18 hr at room temperature. Then the plates were covered with plastic film and stored at 4°C until assay. The assay was performed as follows: the wells were washed three times with 300 µl washing buffer and incubated with 50 µl per well of rabbit anti-CGRP serum at 30°C for 2 hr. The rabbit anti-CGRP serum was diluted 1 : 160,000 in TBS. The wells were washed to remove all unbound anti-CGRP serum. Then the biotinyl-CGRP solution (12 pg/20 µl) and a sample (80 µl) or the CGRP standard solution (80 µl) was applied to each well, followed by standing at 4°C overnight. The CGRP standard solution was made at concentrations of 2–256 pg per 100 µl in Block Ace diluted fourfold in TBS. To remove unbound biotinyl-CGRP, each well was washed 5 times with 300 µl washing buffer. Streptoavidine-alkaline phosphatase solution (100 µl) was added to the wells and incubated for 1 hr at 30°C. To remove unbound streptoavidine-alkaline phosphatase, each well was washed 5 times with 300 µl washing buffer, and 220 µl of AMPPD<sup>TM</sup> solution was added to each well at room temperature. After 15 min of incubation, the chemiluminescent light emission was measured with a LB9501 luminometer (Berthold, Badwildbad, Germany).

**Determination of CGRP released from the mucosa of corpus**

The stomach was excised from rats. The forestomach and antrum were dissected out, and the corpus was inverted (mucosal-side-out). These tissues were rinsed and allowed to stabilize for 1 hr in cold oxygenated modified Krebs-Henseleit solution of the following composition: 110 mM NaCl, 4.7 mM KCl, 1.0 mM CaCl<sub>2</sub>, 1.15 mM MgCl<sub>2</sub>, 25 mM NaHCO<sub>3</sub>, 5.6 mM glucose and 10 mM Hepes (pH 7.4). Following stabilization, these tissues were incubated in test tubes containing 2 ml of modified Krebs-Henseleit solution oxygenated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Capsaicin (final concentration: 1×10<sup>-8</sup>–1×10<sup>-5</sup> M) dissolved in 10% ethanol (0.1 ml) or vehicle alone was added to the incubated tube. Thirty minutes later, the incubated solution was centrifuged at 3,000 rpm for 5 min at 4°C. The CGRP content of the supernatant was determined by CLEIA as described above.

**Capsaicin pretreatment (functional ablation of afferent neurons)**

Defunctionalization of the capsaicin-sensitive afferent nerves was performed according to the previously described method (18). In brief, rats received capsaicin by s.c. injection once daily for three consecutive days (20, 30 and 50 mg/kg) to induce the functional ablation of afferent neurons. All capsaicin injections were done under ether anesthesia, and the rats were pretreated intramuscularly with terbutaline (0.1 mg/kg) and aminophylline (10 mg/kg) to counteract the respiratory impairment associated with capsaicin injection. Defunctionalization of afferent neurons was ascertained by evaluating the reduction of the protective wiping movements in response to intraocular instillation of a 0.1 mg/ml solution of capsaicin. The excised stomach was examined for capsaicin (1×10<sup>-6</sup> M)-induced CGRP release.

**TTX treatment**

Intra-arterial infusion of TTX was performed according to the previously described method (18). After the
induction of anesthesia with urethane (1.2 g/kg, i.p.), the rat was briefly fitted with a tracheal cannula to facilitate spontaneous respiration. A polyethylene catheter was inserted retrogradely into the splenic artery close to the celiac artery for the intra-arterial infusion. The rats received an intra-arterial infusion of TTX (60 ng/min) or saline (0.2 ml/hr) for 30 min. After intra-arterial infusion of TTX, the stomach was excised for the determination of CGRP release. Moreover, TTX (1 × 10⁻⁵ M) was added to the test tube in the determination of capsaicin (1 × 10⁻⁶ M)-induced CGRP release from the stomach.

**Gangliosympathectomy**
Upper abdominal sympathectomy (gangliosympathectomy) was performed by the aid of an operation microscope. The procedure was carried out according to the technique of Kamata et al. (19). The celiac and superior mesenteric ganglia were extirpated. After a postoperative period of 10 days, the stomach was excised and examined for capsaicin (1 × 10⁻⁶ M)-induced CGRP release.

**6-OHDA treatment**
Chemical sympathectomy of rat was induced by intraperitoneal injection of 6-hydroxydopamine (6-OHDA; 150 mg/kg, twice, with a 24-hr interval between each injection) (20). 6-OHDA is a neurotoxin that causes a rapid and complete degeneration of sympathetic nerve terminals after its administration. One week later, the excised stomach was examined for capsaicin (1 × 10⁻⁶ M)-induced CGRP release.

**Statistical analyses**
The results obtained are expressed as means±S.E. Statistical significance was determined by Student’s t-test for the comparison between two groups or one-way analysis of variance (ANOVA) followed by Dunnett’s test or Tukey’s test for multiple comparison where appropriate.

**RESULTS**
The optimal titer of the rabbit CGRP antiserum in this assay was 1 : 160,000 as described in the Materials and Methods, and the CLEIA system has successfully detected CGRP within the range of 2 to 256 pg/well within 1.5 days (Fig. 1).

The basal CGRP release from the isolated stomach was 0.40±0.02 pg/mg wet weight in 30 min of incubation. The application of capsaicin (1 × 10⁻⁸–1 × 10⁻⁵ M) caused a stimulation of CGRP release from the isolated stomach in a concentration-dependent manner (Fig. 2). Capsaicin (1 × 10⁻⁶ M) induced a threefold increase: 1.48±0.13 pg/mg wet weight in 30 min of incubation.

For the TTX experiments, the stomach from the rat pretreated with intra-arterial TTX infusion (60 ng/min for 30 min) was further incubated with TTX (1 × 10⁻⁵ M). The basal levels of CGRP release of the TTX-treated and the normal stomachs were 0.46±0.04 and 0.81±0.18
pg/mg wet weight in 30 min of incubation, respectively. Figure 3 shows that when capsaicin (1 x 10^-6 M) was added to the medium, the basal release was stimulated significantly to the same extents in the TTX-treated and normal stomachs.

In the stomach from the rat with defunctionalization of afferent neurons (pretreatment with high doses of capsai-

**Fig. 3.** Effect of capsaicin on CGRP release from the isolated and TTX-treated stomach. The rats received an intra-arterial infusion of TTX (60 ng/min) or saline (0.2 ml/hr) for 30 min. After intra-arterial infusion of TTX, the rats were sacrificed to excise the stomach. Moreover, TTX (1 x 10^-5 M) was added to the incubated tissue in the determination of CGRP release from the stomach. Capsaicin (1 x 10^-6 M) or vehicle alone was added to the incubated tissue. Thirty minutes later, the CGRP release was determined by CLEIA. Data are expressed as the mean±S.E. of 6 isolated rat stomachs. **P<0.01, significantly different from the control by Tukey's test.

**Fig. 4.** Effect of capsaicin on CGRP release from the isolated stomach obtained from rats with defunctionalization of afferent neurons. Chemical defunctionalization of afferent neurons was performed by consecutive injection of capsaicin (total dose: 100 mg/kg, s.c.) two weeks before the experiment. Capsaicin (1 x 10^-6 M) or vehicle alone was added to the incubated tissue. Thirty minutes later, the CGRP release was determined by CLEIA. Data are expressed as the mean±S.E. of 3-6 isolated rat stomachs. **P<0.01, significantly different from the control by Student's t-test. N.D.: not detected.

**Fig. 5.** Effect of capsaicin on CGRP release from the isolated stomach obtained from sympathectomized rats. Gangliosympathectomy was performed by extirpating the celiac and superior mesenteric ganglia. After a postoperative period of one week at least, the rats were processed for the determination of CGRP release from the stomach. Capsaicin (1 x 10^-6 M) or vehicle alone was added to the incubated tissue. Thirty minutes later, the CGRP release was determined by CLEIA. Data are expressed as the mean±S.E. of 7-8 isolated rat stomachs. **P<0.01, statistically significant difference between two groups by Tukey's test.

**Fig. 6.** Effect of capsaicin on CGRP release from the isolated stomach obtained from 6-OHDA-treated rats. Chemical sympathectomy was induced by intraperitoneal injection of 6-OHDA (150 mg/kg twice with a 24-hr interval) at least 4 days before the experiment. Capsaicin (1 x 10^-6 M) or vehicle alone was added to the incubated tissue. Thirty minutes later, the CGRP release was determined by CLEIA. Data are expressed as the mean±S.E. of 7-8 isolated rat stomachs. **P<0.01, significantly different from the control by Tukey's test.
cin), the levels of the basal and capsaicin (1 × 10⁻⁶ M)-
induced CGRP release were below the limit of detection
(2 pg/well) (Fig. 4). The procedure of upper abdominal
sympathectomy (gangliosympathectomy) did not affect
significantly the basal levels of CGRP; the basal releases
in the sympathectomized and normal stomachs were
0.28 ± 0.04 and 0.48 ± 0.11 pg/mg wet weight in 30 min
of incubation, respectively (Fig. 5). However, after
the stimulation of capsaicin, the levels of CGRP in sympa-
thetomized stomachs were 0.21 ± 0.03 pg/mg wet
weight in 30 min of incubation, being almost the same as
the basal value. After the injection of 6-OHDA, the basal
and capsaicin (1 × 10⁻⁶ M)-stimulated CGRP levels were
0.44 ± 0.06 and 1.26 ± 0.12 pg/mg wet weight in 30 min
of incubation, respectively. There was no difference be-
tween 6-OHDA treated and normal stomachs (Fig. 6).

DISCUSSION

The present study shows that the release of CGRP from
the isolated rat stomach was measurable, and the new
CLEIA system, which we developed, has successfully
detected the released CGRP within 1.5 days. When com-
pared to the conventional RIA system (21), the time
required for the assay was reduced to approximately half
for the same sensitivity. Recent progress in chemi-
luminescence technology (22, 23) has made it possible
to obtain the quantum yield of generating light at the
rate of 0.10 photon per min (signal as photon=1
molecule × 0.10 × turnover of enzyme), while the use of
¹²⁵I as the radioisotope requires 7.5 × 10⁶ atoms to gener-
ate 1 photon per min (24). EIA using a chemiluminescent
substrate is now for superior to RIA in terms of sensi-
tivity. Bronstein et al. (25) reported that AMPPDTM,
a chemiluminescent substrate for alkaline phosphatase,
when used in immunoassays permits the detection of
10⁻⁴⁰ moles of enzyme. Furthermore, the EIA method is
more easily performed than the RIA method, because it
avoids the radiation hazards encountered during labeling
and disposal of wastes. Moreover, if rabbit anti-CGRP
serum and biotinyl-CGRP were substituted by other ap-
propriate agents, this CLEIA system might be applicable
for assays of various other endogenous biological sub-
stances.

The capsaicin-sensitive afferent nerve has been recently
reported to play an important role in gastroprotection
(11–14). The peripheral capsaicin-sensitive nerve has
been shown to release a CGRP, which is known to protect
gastric mucosa against noxious stimuli (17). From these
aspects, it seemed very important to directly determine
the levels of CGRP released from the gastric wall. There-
fore, we measured capsaicin-induced CGRP release
from the isolated and inverted rat stomach. The forestomach
was dissected out from the isolated stomach, and then the
stomach was inverted (musosal-side-out). Since the in-
verted stomach shrank and became sac-shaped, it would
seem difficult for capsaicin to reach the serosal side. Fur-
thermore, the serosa and muscle layers remain intact,
meaning that CGRP release through these layers into the
medium is rather more difficult than through the mu-
cosa. For the reasons mentioned above, it would be pos-
sible to assume that the measurable CGRP was mostly
derived from the released CGRP from the mucosal side.
Our results showed that the application of capsaicin in the
isolated stomach was able to release CGRP in a concen-
tration-dependent fashion and are in agreement with
those of Ren et al. (26) obtained from the isolated rat an-
trum. In our study, there was significant CGRP release at
a relatively low concentration of capsaicin (1 × 10⁻⁰⁷ M),
suggesting that the mucosa of the corpus is more sensitive
to capsaicin than the antrum. Since the levels of the basal
and capsaicin-induced CGRP release were below the limit
of the detection in the stomach from the rat with defunc-
tionalization of afferent neurons (pretreatment with high
doses of capsaicin), CGRP proved to be a transmitter of
capsaicin-sensitive nerves in accordance with previous
reports (12–16, 18). Our results suggested that CGRP
was released to the gastric lumen from the gastric mu-
cosa. On the other hand, capsaicin-induced CGRP re-
lease was even observed in the isolated stomach incubated
with TTX from the rat to which intra-arterial TTX was
previously infused. Other investigators have shown that
neuropeptide release from peripheral sensory nerves in-
volves both TTX-sensitive and TTX-resistant compo-
nents (12, 26). Our study indicated that the capsaicin-in-
duced CGRP release from the isolated stomach is mostly
attributable to the TTX resistant component, suggesting
the release of the stored CGRP from sensory nerve ter-
inals. These results indicate that the direct determina-
tion of released CGRP from the stomach might be useful
in studying the defensive mechanisms of capsaicin-sensi-
tive nerves.

Pharmacological reports have shown that acute mu-
coal application of capsaicin protected against mucosal
damage induced by various necrotizing agents and caused
gastric mucosal vasodilation (27, 28). This capsaicin-in-
duced hyperemia is abolished by pretreatment with high
doses of capsaicin and inhibited by intra-arterial infusion
of human CGRP8–37, a CGRP-receptor antagonist (18).
The mucosal hyperaemia of afferent nerve stimulation
was reportedly significantly inhibited by perineural appli-
cation of capsaicin to the celiac/superior mesenteric gan-
glia, but not to the vagus nerves (29). Studies combining
neuronal tract tracing with immunocytochemistry have
shown that up to 95% of spinal afferent neurons in-
ervating the stomach contain CGRP immunoreactivity,
Kawasaki et al. (33) reported that there is a possible interaction between noradrenergic and CGRP containing nerves in rat mesenteric vascular beds. Since the procedure of gangliosympathectomy clearly abolished capsaicin-induced CGRP release from the isolated stomach in our results and the terminals of noradrenergic nerves reportedly exist in gastric wall (34), it might be possible to speculate that if the interaction between noradrenergic and CGRP containing nerves occurs also in the gastric wall, the long-term decrease of activity of noradrenergic nerves after gangliosympathectomy would affect the activity of CGRP-containing nerves, resulting in abolition of CGRP release in response to capsaicin. Therefore, we used 6-OHDA, a neurotoxin for noradrenergic nerves, for chemical depletion of noradrenaline in the gastric wall to investigate whether there is such a relationship in the gastric wall. However, 6-OHDA, a neurotoxin that causes a rapid and complete degeneration of adrenergic nerve terminals, did not affect the increase in CGRP levels after exposure to capsaicin, suggesting that the CGRP release is not regulated by adrenergic nerves. Therefore, the fact that capsaicin-induced CGRP release was abolished by the procedure of gangliosympathectomy shows that capsaicin-sensitive nerves run through the celiac/superior ganglia and project to the gastric wall. Since the experiment was carried out about 1 week after gangliosympathectomy, it is possible to assume that the peripheral terminals of capsaicin nerves would degenerate. On the other hand, it is also accepted that the majority of cell bodies of CGRP-containing afferent nerves supplying the stomach were located in dorsal root ganglia (31). Taking these facts into consideration, these findings suggest that capsaicin-sensitive nerves, particularly the CGRP containing nerves, supplying the stomach mainly originate from the dorsal root ganglia through the celiac/superior ganglia. From the standpoint of direct CGRP release from the stomach, our results further confirmed that the CGRP-containing nerves projecting to the gastric wall runs mostly in the sympathetic nerve trunk.

In conclusion, the CLEIA system, which we developed, may be useful for detecting the released CGRP and studying the activity of capsaicin-sensitive nerves, particularly the CGRP-containing nerves. Our results also showed that capsaicin-sensitive nerves in the gastric wall discharge CGRP and that the released CGRP originates from the nerve running in the sympathetic nerve trunk.

REFERENCES

1. Amara SG, Jonas V, Rosenfeld MG, Ong ES and Evans RM: Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature 298, 240–244 (1982)
2. Tschopp FA, Tobler PH and Fischer JA: Calcitonin gene-related peptide in the human thyroid, pituitary and brain. Mol Cell Endocrinol 36, 53–57 (1984)
3. Kawai Y, Takami K, Shiosaka S, Emson PC, Hillyard CT, Girgis S, MacIntyre I and Tohyama M: Topographic localization of calcitonin gene-related peptide in the rat brain: An immunohistochemical analysis. Neuroscience 15, 747–763 (1985)
4. Gibson SJ, Polak JM, Bloom SR, Sabati IM, Mulderry PM, Ghatei MA, McGregor GP, Morrison JFB, Kelly JS, Evans RM and Rosenfeld MG: Calcitonin gene-related peptide immunoreactivity in the spinal cord of man and eight other species. J Neurosci 4, 3101–3111 (1984)
5. Scalfit G and Jacobowitz DH: Calcitonin gene-related peptide: Detailed immunohistochemical distribution in the central nervous system. Peptides 6, 721–745 (1985)
6. Okimura Y, Chihara K, Abe H, Kita T, Kashio Y, Sato M and Fujii T: Calcitonin gene-related peptide-like immunoreactivity in the central nervous system and peripheral organs of rat. Regul Pept 17, 327–337 (1987)
7. Franco-Cereceda A, Henke H, Lundberg JM, Petermann JB, Hökfelt T and Fisher JA: Calcitonin gene-related peptide (CGRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: Distribution and release by capsaicin. Peptides 8, 399–410 (1987)
8. Holzer P: Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 24, 739–768 (1988)
9. Maggi CA and Meli A: The sensory-efferent function of capsaicin-sensitive sensory neurons. Gen Pharmacol 19, 1–43 (1988)
10. Limlomwongse L, Chaitauchawong C and Tongyai S: Effect of capsaicin on gastric acid secretion and mucosal blood flow in the rat. J Nutr 109, 773–777 (1979)
11. Holzer P, Pabst MA, Lippe IT and Peskar PB: Afferent nerve-mediated protection against deep mucosal damage in the rat stomach. Gastroenterology 98, 838–848 (1990)
12. Holzer P and Lippe IT: Stimulation of afferent nerve ending by intra gastric capsaicin protects against ethanol-induced damage of gastric mucosa. Neurosciences 27, 981–987 (1988)
13. Holzer P, Pabst MA and Lippe IT: Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa. Gastroenterology 96, 1425–1433 (1989)
14. Uchida M, Yano S and Watanabe K: The role of capsaicin sensitive nerves in protective effect of capsaicin against absolute ethanol-induced gastric lesions in rats. Jpn J Pharmacol 55, 279–282 (1991)
15. Sternini C, Reeve JR and Brecha N: Distribution and characterization of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats. Gastroenterology 93, 852–862 (1987)
16. Holzer P and Sametz W: Gastric mucosal protection against
ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons. Gastroenterology 91, 975–981 (1986)

17 Lippe IT, Lorbach M and Holzer P: Close arterial infusion of calcitonin gene-related peptide into the rat stomach inhibits aspirin- and ethanol-induced hemorrhagic damage. Regul Pept 26, 35–46 (1989)

18 Onodera S, Shibata M, Tanaka M, Inaba N, Yamaura T and Ohnishi H: Gastroprotective activity of FRG-8813, a novel histamine H2-receptor antagonist, in rats. Jpn J Pharmacol 68, 161–173 (1995)

19 Kamata K, Watanabe M and Kasuya Y: Changes in the response to drugs of the rat stomach after a chronic vagotomy and a sympathectomy. Folia Pharmacol Jpn 74, 225–238 (1978) (Abstr in English)

20 Graffner H, Ekelund M, Hakanson R and Rosengren E: Effect of different denervation procedures on catecholamines in the gut. Scand J Gastroenterol 20, 1276–1280 (1985)

21 Seth R, Zaidi M, Fuller JQ and Self CH: A highly specific and sensitive-immunometric assay for calcitonin gene-related peptide based on enzyme amplification. J Immunol Methods 111, 11–16 (1988)

22 Brundett RB and White EH: Synthesis and chemiluminescence of luminol and isoluminol. J Am Chem Soc 96, 7497–7502 (1974)

23 Valkero G and Barton R: Immunoconcentration™—a new format for solid-phase immunoassays. Clin Chem 31, 1427–1431 (1985)

24 Bounaud JY, Bounaud MP and Begon H: One-step chemiluminescent immunoassay of free thyroxin with acridinium-ester-labeled thyroxin evaluated and compared with a two-step radioimmunoassay. Clin Chem 34, 2556–2560 (1988)

25 Bronstein I, Edwards B and Voyta CJ: 1,2-Dioxetanes: Novel chemiluminescent enzyme substrates. Applications to immunoassays. J Biolum Chemilum 4, 99–111 (1989)

26 Ren J, Young RL, Lassiter DC and Harty RF: Calcitonin gene-related peptide mediates capsaicin-induced neuroendocrine responses in rat antrum. Gastroenterology 104, 485–491 (1993)

27 Takeuchi K, Nida H, Matsumoto J, Ueshima K and Okabe S: Gastric motility changes in capsaicin-induced cytoprotection in the rat stomach. Jpn J Pharmacol 55, 147–155 (1991)

28 Merchant NB, Dempsey DT, Grabowski MW, Rizzo M and Ritchie WP: Capsaicin-induced gastric mucosal hyperemia and protection: The role of calcitonin gene-related peptide. Surgery 116, 419–425 (1994)

29 Raybould HE, Sterrini C, Eysselle VE, Yoneda M and Holzer P: Selective ablation of spinal afferent neurons containing CGRP attenuates gastric hyperemic response to acid. Peptides 13, 249–254 (1992)

30 Green T and Dockray GJ: Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea pig. Neuroscience 25, 181–193 (1988)

31 Sterrini C and Anderson K: Calcitonin gene-related peptide-containing neurons supplying the rat digestive system: Differential distribution and expression pattern. Somatosens Mot Res 9, 45–59 (1992)

32 Su HC, Bishop AE, Power RF, Hamada Y and Polak JM: Dual intrinsic and extrinsic origins of CGRP- and NPY-like immunoreactive nerves of rat gut and pancreas. J Neurosi 7, 2674–2687 (1990)

33 Kawasaki H, Nuki C, Saito A and Takasaki T: Adrenergic modulation of calcitonin gene-related peptide-containing nerves in the vascular adrenergic neurotransmission. Brain Res 506, 287–290 (1990)

34 Nakamura M, Watanabe N, Tsukada N, Oda M and Tsujiya M: Demonstration of the adrenergic nerves in the rat gastric mucosa—A histofluorescence and electron microscopic study in comparison with the distribution of the cholinergic nerves. Okajima Folia Anat Jpn 59, 65–86 (1982)