Central Autonomic Activation by Intracisternal TRH Analogue Excites Gastric Splanchnic Afferent Neurons

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Adelson, David W., Jen Yu Wei, Mahrokh Yashar, T. J. O-Lee, and Yvette Tache. Central autonomic activation by intracisternal TRH analogue excites gastric splanchnic afferent neurons. J. Neurophysiol. 81: 682–691, 1999. Intracisternal (ic) injection of thyrotropin-releasing hormone (TRH) or its stable analogue RX 77368 influences gastric function via stimulation of vagal muscarinic pathways. In rats, the increase in gastric mucosal blood flow evoked by a low ic dose of RX 77368 occurs via release of calcitonin gene-related peptide from capsaicin-sensitive afferent neurons, most probably of spinal origin. In this study, the effect of low ic doses of RX 77368 on afferent impulse activity in splanchnic single fibers was investigated. The cisterna magna of overnight-fasted, urethane-anesthetized Sprague-Dawley rats was acutely cannulated, and fine splanchnic nerve twigs containing at least one fiber responsive to mechanical probing of the stomach were isolated at a site immediately distal to the left suprarenal ganglion. Unit mechanoreceptive fields were encountered in all portions of the stomach, both superficially and in deeper layers. Splanchnic afferent unit impulse activity was recorded continuously during basal conditions and in response to consecutive ic injections of saline and RX 77368 (15–30 min later; 1.5 or 3 ng). Basal discharge rates ranged from 0 to 154 impulses/min (median = 10.2 impulses/min). A majority of splanchnic single units with ongoing activity increased their mean discharge rate by ≥20% after ic injection of RX 77368 at either 1.5 ng (6/10 units; median increase 63%) or 3 ng (19/24 units; median increase 175%). Five units lacking impulse activity in the 5-min before ic RX 77368 (3 ng) were also excited, with the onset of discharge occurring within 1.0–5.0 min postinjection. In units excited by ic RX 77368, peak discharge occurred 15.6 ± 1.3 min after injection and was followed by a decline to stable activity levels ±20–40 min thereafter. In a few cases (4/24), ic RX 77368 (3 ng) inhibited the impulse activity of initially active units, with a time course comparable to that seen in units excited by the same treatment. The pattern of discharge in most units was not suggestive of mechanical modulation of activity by rhythmic gastric contractions. The data demonstrate that low ic doses of TRH analogue induce sustained increases in afferent discharge in a substantial proportion of splanchnic neurons innervating the rat stomach. These findings support the notion that splanchnic afferent excitation occurs concomitantly with vasodilatory peptide release from gastric splanchnic afferent nerve terminals after ic TRH-induced autonomic activation.

INTRODUCTION

The most widely recognized sensory function of the splanchnic nerve is the mediation of abdominal pain (Cervero 1994). In addition, splanchnic afferent neurons are known to mediate autonomic reflexes, such as gastric motility-inhibiting reflexes in response to antral (Abrahamsson and Glise 1984) or duodenal (Holzer and Raybould 1992) distention and pseudoadaptive cardiovascular responses to noxious stimuli (Huang and Longhurst 1994; Rozsa et al. 1988), as well as to participate in behavioral thermoregulatory responses to intra-abdominal temperature changes (Rawson and Quick 1972). Previous electrophysiological studies of splanchnic afferent function have focused on responses to peripherally applied stimuli (Adelson et al. 1997a; Floyd and Morrison 1974; Haupt et al. 1983; Longhurst et al. 1984; Pan and Longhurst 1996). In contrast, the responses of spinal abdominal afferent neurons to physiological changes in the viscera resulting from central autonomic activation have not been previously studied electrophysiologically.

A variety of peptides act in the brain stem to influence visceral physiology via the modulation of autonomic nervous system activity (Brown and Tache 1981; Tache et al. 1990; Tache and Yang 1990). Among the best studied of this type of brain–gut interaction is the vagal regulation of gastric function by medullary thyrotropin-releasing hormone (TRH) (Tache et al. 1993). The dorsal motor nucleus of the medulla (DMN) receives a rich supply of TRH-immunopositive fibers projecting from the medullary raphe nuclei and contains an abundance of neurons expressing the TRH-receptor (Lynn et al. 1991; Manaker and Rizio 1989). TRH-immunopositive nerve terminals make asymmetric synaptic contacts on the dendrites of gastric vagal motoneurons (Rinaman and Mishelis 1990), and TRH directly evokes excitatory postsynaptic potentials from preganglionic vagal neurons in the DMN (Raggenbass et al. 1990). TRH, or its stable analogue RX 77368, microinjected into the DMN or introduced into the medullary cerebrospinal fluid (CSF) by intracisternal (ic) injection, stimulates gastric function via vagal cholinergic pathways (Garrick et al. 1989; Kato et al. 1995, 1996; Kiraly et al. 1993, 1998; Maeda-Hagiwara and Tache 1987; Stephens et al. 1988; Tache 1988; Tache et al. 1989; Thieffin et al. 1989; Yoneda and Tache 1993). The role of medullary TRH in the modulation of gastric function has been further substantiated by demonstrations that TRH antibody or TRH-receptor antisense oligodeoxynucleotides injected ic or microinjected into the dorsal vagal complex (DVC) block vagally mediated changes in gastrointestinal function evoked by stimuli such as cold restraint, sham feeding, chemical...
excitation of raphe pallidus neurons, or intravenous 2-deoxy-D-glucose (Barrachina et al. 1997; Garrick et al. 1994; Kaneko et al. 1995; Kaneko and Tache 1995; Martinez et al. 1998; Okumura et al. 1995a,b; Yang et al. 1993). Intracisternal injection of low doses of the stable TRH-analogue RX 77368 (1.5 ng) in urethan-anesthetized rats significantly increases vagal efferent discharge (O-Lee et al. 1997), gastric mucosal blood flow (GMBF), and systemic blood pressure, while reducing gastric mucosal vascular resistance (Kiraly et al. 1997). Gastric hyperemia in response to centrally administered TRH occurs via vagal cholinergic pathways, and is not related to changes in luminal acid secretion (Kiraly et al. 1997; Tanaka et al. 1997; Thie®n et al. 1989) but occurs via the release of the vasodilatory neuropeptide calcitonin gene-related peptide (CGRP) from capsaicin-sensitive primary afferent neurons (Kiraly et al. 1994, 1997). Because splanchnic afferent neurons are the major extrinsic neural source of rat gastric CGRP (Green and Dockray 1988), these data suggested that splanchnic afferent impulse discharge might also be recruited by ic TRH. The aim of this study was to assess afferent impulse activity in splanchnic nerves innervating the stomach in response to central autonomic activation resulting from ic injection of RX 77368 at doses that are known to evoke gastric hyperemia via CGRP release from capsaicin-sensitive neurons. As in prior functional studies (Kiraly et al. 1997), RX 77368 was used in preference to the natural ligand because the dimethylated N-terminal prolineamide renders the compound resistant to degradation by TRH-catabolizing enzymes such as proline endopeptidase and pyroglutamyl aminopeptidase (Grif®ths et al. 1982), whose activity levels cannot easily be controlled or assayed. Although it binds rat brain TRH receptor with a three- to fourfold lower affinity than TRH (Sharif et al. 1991), ic RX 77368 causes more potent and longer-lasting vagal efferent excitation than does the natural ligand at comparable doses (O-Lee et al. 1997), presumably because of its resistance to catabolism, as noted in other systems (Metcalf et al. 1981).

METH O D S

Animals

Male Sprague Dawley rats (Harlan Laboratories, San Diego, CA) weighing 250–350 g were housed under conditions of controlled temperature (20 ± 3°C) and illumination (12 h light cycle beginning at 6 a.m.). Rats were provided Purina Laboratory Chow (Ralston, Purina, St. Louis, MO) and tap water ad libitum. Animals were fasted, with free access to drinking water, 16–24 h before the experiments. Rats were anesthetized by im (hindlimb) injection of 25% urethan (1.5 g/kg) 30–60 min before the start of surgery. An additional dose of 0.1–0.25 g/kg ip was given immediately before surgery if, as was usually the case, the corneal reflex was intact. Adequate depth of anesthesia was determined at regular intervals by the absence of response to ear-pinch and corneal reflex. A temperature-controlled heating block was used to maintain rectal temperature at a stable temperature in the range 35.5–37.5°C throughout the experiment. All procedures performed were approved by the VA West Los Angeles Animal Research Subcommittee.

Drugs

RX 77368 [γ-Glu-His-(3,3′-dimethyl)-Pro-NH₂, Ferring Pharmaceuticals, Feltham, Middlesex] was stored in a stock solution (10 ng/μl, 0.1% bovine serum albumin/saline) at −70°C. Two-microliter aliquots of the stock solution were made biweekly and kept at −20°C until the time of the experiment. Aliquots were diluted in sterile, pyrogen-free 0.9% saline (Sigma, St. Louis, MO) to a final concentration of 1.5 or 3 ng/10 μl immediately before use. The same saline solution was used for control ic injections.

Surgeries

CANNULATION OF THE CI STERN A MAGNA. The method was as previously described (Kiraly et al. 1994). The head of the rat was fixed in a stereotaxic holder (Kopf, Model 900), and the atlantooccipital membrane was exposed. Approximately 1.5 mm caudal to the edge of the occipital bone, a 25-g needle was inserted perpendicularly into the membrane to two-thirds the depth of its bevel, and removed. A PE-10 polyethylene catheter (Intramedic, Becton Dickinson, Parsippany, NJ; length: 6 cm) connected to an injection port comprising a short length of stainless steel tubing connected to PE-20 polyethylene tubing (total dead space of tubing + port: 5 μl) was inserted through the hole into the cisterna magna. Successful cannulation was verified by leakage of clear CSF from the catheter. If blood was observed in the CSF, the experiment was abandoned. A drop of cyanoacrylate glue (Krazy Glue, Itasca, IL) was used to hold the cannula in place. After the glue dried, it was covered with cotton and the incision was sutured closed. The cannula was capped to prevent leakage.

TRACHEAL AND VN CANNULAE. After cannulation of the cisterna magna, tracheotomy was performed to facilitate ventilation. Additionally, a PE-50 cannula was inserted into the left jugular vein, and sterile, pyrogen-free 0.9% saline (Sigma) was continuously infused at a rate of 0.3 ml/h with a syringe pump (Sage Instruments, model 341) to maintain hydration.
ELECTROPHYSIOLOGICAL PREPARATION. Laparotomy was performed, and the skin flaps were secured to a stainless steel ring, exposing the abdominal viscera. All exposed surfaces were kept moist by covering with cotton soaked in warm 0.1 M phosphate-buffered (pH 7.4) saline (Sigma). The spleen and stomach were displaced gently rightward and rostrally to expose the left kidney and the celiac–superior mesenteric ganglionic complex and were held in place with a plastic holder (Fig. 1). In this arrangement, the dorsal aspect of the entire stomach was accessible for mechanical probing to locate gastric mechanosensitive receptive fields while the ventral surface could be partially explored. The left suprarenal ganglion was located, and the splanchnic nerve was traced distally, toward the celiac ganglion. One of the two more medial splanchnic branches exiting the suprarenal ganglion (Fig. 1) was gently freed from surrounding tissue several millimeters distal to the suprarenal ganglion and placed on a small dissecting plate, without severing the nerve, and warm mineral oil was layered over the nerve. In most cases, the more lateral (and larger) of these two medial branches was used (Fig. 1, branch 1). No notable differences were apparent in the distribution of gastric receptive fields or responsiveness to mechanical stimuli or IC RX 77368 between units from these two splanchnic branches.

Using a dissecting microscope (Olympus SZ30) for observation and fine forceps, a “picking window” was opened in the epineurial and perineurial sheaths from the exposed surface of the nerve for a length of ~2 mm, and fine nerve twigs (5- to 15-μm diameter, 1–2 mm in length) were gently separated from the nerve trunk. The distal cut end of an isolated twig was placed on one electrode of a bipolar platinum wire electrode (wire diameter: 25 μm) immersed in the mineral oil pool, and a strand of connective tissue was attached to the other electrode.

ELECTROPHYSIOLOGICAL RECORDING. Impulse activity was fed from the electrode into an isolated biological amplifier (ISO-DAM 6, World Precision Instruments, Sarasota, FL, modified by the manufacturer to provide specific band-pass filtering capabilities) and amplified 100-fold with a passband set at 100 Hz to 10 kHz. The signal was subsequently amplified 250- to 500-fold to give peak–peak waveform amplitudes between 1 and 5 V. Traces were simultaneously recorded. No digital differential switches (DTC-700 DAT deck) and acquired onto a personal computer equipped with an A/D board (DT2831, Data Translation, Marlboro, MA) using the acquisition module of the WAVEFORM impulse analysis software suite for MS-DOS (Adelson et al. 1996). Stimulus application was marked with a foot pedal on a separate channel.

Experimental procedures

ISOLATION OF SPLANCHNIC FILAMENTS WITH GASTRIC MECHANORECEPTIVE FIELDS. For each splanchnic filament isolated, impulse activity was recorded for several minutes to determine whether the filament contained sufficiently few (typically 0–3) units with stable, easily distinguishable waveforms. A blunt glass rod was then used to search for gastric mechanoreceptive fields, first with gentle stroking of the surface of the accessible portions of the stomach and subsequently by pressing the stomach more firmly. If no unit in the filament responded to this probing or if too many units were active to allow a reliable sorting of waveforms, the filament was abandoned and another was picked. Typically, fewer than five filaments had to be isolated before one containing a fiber with a gastric mechanoreceptive field was found. In most cases the nerve remained predominantly intact by the time an appropriate filament was chosen, although in some instances, because of difficulties with the dissection, the nerve was largely or completely interrupted. On the basis of the number of units sampled, no differences were apparent in responses from preparations with intact versus interrupted left celiac–splanchnic nerves. Once at least one unit in the filament was activated by gastric probing, further exploration was avoided to minimize disturbance of the tissue and of the recording conditions. Receptive fields of other units were sought at the end of the experimental protocol described subsequently.

Experimental protocols

RECORDING OF BASAL ACTIVITY. After isolation of a filament, there was typically a period of 10–30 min during which ongoing activity subsided steadily. Once an appropriate splanchnic twig was isolated, discharge was allowed to stabilize. The WAVEFORM acquisition software reports the number of impulses (whose peak amplitude exceeds a user-determined amplitude threshold) per minute, as well as the mean and SDs in discharge per minute for each 5-min interval. When mean discharge in two successive 5-min intervals did not differ by more than 1 SD, drug testing was initiated.

RESPONSES TO IC INJECTIONS. First, 15 μl of saline was slowly (30 s) injected via Hamilton syringe into the ic cannula. After a period ranging from 15 (in early experiments) to 30 (most experiments) min, 1.5 or 3 ng of RX 77368 in 10 μl was injected with a separate Hamilton syringe, followed by a flush of 5 μl saline, over 10 s. In some experiments, subsequent injections of RX 77368, either with or without a preceding second ic saline injection, were made at 60- or 90-min intervals.

LOCALIZATION OF RECEPTIVE FIELDS. At the end of the experiment, a more thorough search was conducted to locate gastric mechanoreceptive fields for as many of the units recorded during the experiment as possible. Receptive fields were classified as located in the antrum, glandular stomach, or nonglandular stomach. Units that could be activated by light stroking of the gastric serosa were identified as having superficial receptive fields. The inability to activate a unit by light stroking did not necessarily rule out the presence of a serosal receptive field because certain portions of the gastric serosa were inaccessible.

The limited access to the recording area precluded the determination of conduction velocities for the units studied. However, in the rat the splanchic afferent population derives almost exclusively from CGRP-expressing small sensory ganglion cells (Kashiba et al. 1991), and the splanchic contribution to the mesenteric nerves is entirely unmyelinated (Cervero and Sharkey 1988). The duration of all impulse waveforms recorded was consistent with that of C-fibers recorded from rat splanchnic nerve in vitro (Adelson et al. 1996, 1997a).

Data analysis

Raw impulse activity records were sorted into single-unit processed data files with the sorting module of the WAVEFORM analysis software. Any units that could not reliably be sorted throughout the duration of the experiment were rejected. For each stimulus, the discharge during the 5 min before ic injection of either saline or RX 77368 was calculated with the graphing module of the WAVEFORM software, along with the peak 5-min discharge after ic injections. Statistical tests were performed with SigmaStat for Windows Version 2.03 (SPSS, Chicago, IL). Differences were considered statistically significant for P < 0.05. Specific tests used to compare data are indicated in the text. All activity plots were output from WAVEFORM software.

RESULTS

A total of 50 single units from 19 animals analyzed in detail were characterized in terms of their responses to con-
successive ic injections of saline and of RX 77368 (1.5 or 3 ng) as well as their level of ongoing activity and, where possible, receptive field location and responses to mechanical perturbation of their receptive fields. Of these 50 units, 2 had receptive fields on the pancreas and were not excited after ic saline or ic RX 77368 injection. Four units did not begin discharging until long after RX 77368 injection, would not have been recognized had recording been stopped within the normal interval after the first RX 77368 injection, and so were not tallied. Four units were only excited briefly with peak discharge occurring \( \approx 5 \text{ min} \) after either ic saline or ic RX 77368. This discharge was considered an artifact of ic injection in these units. One unit was excited briefly after ic RX 77368 (3 ng) but not after ic saline, but the time course of its response was different from all other units responding to RX 77368, resembling instead the brief responses to either ic saline or RX 77368 injection in those four units responding to both. The results presented refer to the remaining 39 units. In addition to the units analyzed, most nerve twigs contained one or more single units that fired only a few impulses, not correlated to application of any stimulus, during the course of extended \((>2 \text{ h})\) observations.

**Responses to ic injections**

Ongoing (basal) discharge among the units studied ranged from 0 to 154 impulses/min \( (\text{median} = 10.2 \text{ impulse/min}) \). In the great majority of units, impulse activity in any 5-min period after ic saline did not differ from that immediately before ic saline by \( >10\% \). In four units, however, slow trends in discharge, random bursts (no obvious relation to the ic injection and not different from occasional episodes in units before or long after stimulation), or a combination of low initial mean impulse activity and random fluctuation in activity yielded peak changes in discharge after saline of \( \approx 70\% \) relative to the 5 min before injection. In addition, two units appeared to increase their ongoing discharge as a result of ic saline injection. In all six of these cases, however, the peak response to ic RX 77368 was two- to fivefold greater than that to ic saline and included the distinct rise in activity followed by a return to stable levels that was characteristic of RX 77368-induced responses in other units. In several experiments in which it was tested, a second ic saline injection given \( >60 \text{ min} \) after ic RX 77368 did not evoke a change in unit impulse activity.

The percent increase from basal to peak discharge after ic RX 77368 for the population of all units with ongoing activity \((>5 \text{ impulses/min})\) was significantly greater than the equivalent change after ic saline \( (\text{Wilcoxon Signed Rank test}) \) at both the 1.5- \((n = 8, P = 0.039)\) and 3-ng \((n = 24, P = 0.017)\) doses.

Figure 2A shows the discharge \((\text{impulses/5 min})\) at the point of maximum difference from basal discharge after ic RX 77368 plotted versus basal discharge for each unit studied. Several patterns of response to ic RX 77368 were observed. A majority of units at each dose increased mean firing rates by \( >20\%, \) and the remainder were inhibited or not excited after ic RX 77368 \((\text{Fig. 2A})\). The percent increase among units excited by ic RX 77368 and having discharge rates \( 5-50 \text{ impulses/min} \) before peptide injection \( (\text{the span covered by units excited at the 1.5-ng dose}) \) was \( 56 \pm 15\% \) and \( 253 \pm 106\% \) at 1.5- and 3-ng doses, respectively \((\text{Fig. 2B})\). Among the entire population of units with ongoing discharge in the 5 min before peptide injection, 3 ng RX 77368 ic excited 19/24 units \( (\text{median increase 175\%}) \), inhibited 4/24 units \( (\text{median decrease 46\%}) \), and had no effect on 1 unit. In addition, five units lacking discharge in the 5 min before 3 ng ic RX 77368 were activated by this stimulus; two of these were completely silent before testing, whereas three were active after gastric probing but ceased firing before testing with RX 77368. After 1.5 ng RX 77368 ic, 6 of 10 units were excited \( (\text{median increase 63\%}) \), and 4 of 10 units did not respond. The proportion of units with ongoing activity that were excited by RX 77368 at 1.5 versus 3 ng did not differ significantly \( (P = 0.39, \text{Fisher exact test}) \).

The time course of ic RX 77368-induced excitation was more or less stereotypical, comprising an increase in impulse activity beginning within 10 min postinjection, reaching a peak \( 15.6 \pm 1.3 \text{ min} (n = 30) \) postinjection, and declining over the course of the following 20-40 min to a stable discharge level usually at or below that immediately preceding peptide injection \((\text{Fig. 3, A-C})\). In some cases discharge stabilized at a level greater than pretreatment values \( (e.g., \text{Fig. 3B})\). The five units excited by ic RX 77368 (3 ng) that lacked discharge in the 5 min before peptide injection provided the opportunity to discern the minimum time required for this stimulus to effect changes in splanchnic afferent unit discharge. In these units, the first discharge occurred \( 2.6 \pm 0.8 \text{ min} \) \((\text{range: 1.0-5.0 min})\) after ic RX 77368 injection. In units inhibited by ic RX 77368 \((n = 4)\), the minimum in 5-min discharge occurred \( 21.3 \pm 6.2 \text{ min} \) postinjection and recovered over a time course similar to that of units excited by the same treatment \((\text{Fig. 3D})\).

In most cases \((23/30, 76\%)\) the pattern of discharge evoked by ic RX 77368 was irregular \((\text{Fig. 4A})\), lacking any recognizable periodicity or at best weak periodicity. In four units, irregular discharge after ic RX 77368 was interrupted with intermittent bursts of activity \((\text{Fig. 4B})\), whereas in two units a distinct periodicity was discernible in the pattern of discharge \((\text{Fig. 4C})\) during an interval lasting \( >10 \text{ min} \) surrounding the period of peak discharge. Units with either bursting or periodic activity were among those with the greatest peak responses to ic RX 77368, including the three most active units. Discharge in one unit included occasional short volleys of activity both before and after ic RX 77368.

**Responses to mechanical stimulation**

The responses evoked from gastric splanchnic units by mechanical stimulation varied from a single volley of impulses during the stimulus with no afterdischarge to elevated irregular discharge with extended afterdischarge. In some units, a period of silence lasting \( \approx 1 \text{ min} \) followed the cessation of stimulation, with subsequent increased afterdischarge that decayed over a period of several minutes \((\text{Fig. 5A})\). In other units, no period of silence was observed, whereas in some, discharge continued to increase even after the cessation of the stimulus, subsequently decaying over the following minutes \((\text{Fig. 5C})\). The longest period of afterdischarge observed lasted \( >18 \text{ min} \), although afterdischarge in most
units lasted between 1 and 3 min. Two units could only be activated by mechanical probing after sustained firm pressing of the stomach with the glass rod.

Receptive fields in all regions of the stomach were identified. Of 19 units whose receptive fields were localized, 3 had receptive fields in the antrum, near or at the point of pancreatic attachment, 4 were found in the central portion of the glandular stomach, 6 more were localized on or adjacent to the dividing ridge between glandular and nonglandular stomach, and 6 were excited by probing the nonglandular stomach. Three units, one in the antrum and two in the central portion of the glandular stomach, responded to light touch of the stomach surface and were deemed to have superficial receptive fields. In some cases, the deformation of the nonglandular stomach certainly perturbed the glandular stomach as well, although less so than direct probing of the glandular stomach. The discharge evoked by ic RX 77368 was not discernibly correlated to either the pattern of response to mechanical stimulation, the location of the receptive field, or whether the receptive field appeared superficial or deep.

**DISCUSSION**

These data demonstrate that low doses of the stable TRH analogue RX 77368 injected ic cause excitation of splanchnic afferent fibers with gastric receptive fields. At a 3-ng dose, 78% of splanchnic units with ongoing activity were excited by ic RX 77368, as were five initially silent units. Mechanoreceptive fields of units excited by ic RX 77368 were found in all regions of the stomach. The relatively large number of units with receptive fields identified in the nonglandular stomach may be a reflection of bias caused by the physical accessibility of this portion of the stomach given its positioning, which precluded an exhaustive and identical exploration of receptive fields from all regions. Units that could be excited by lightly touching the serosal surface of the stomach and units that could only be mechanically excited by substantial indentation of the stomach were activated by ic RX 77368, indicating that fibers innervating superficial and deep layers were surveyed. In most cases the response to mechanical stimulation appeared consistent with a direct effect of physical perturbation, although elevated afterdischarge lasting ≥1 min was common. However, in at least two cases, discharge provoked by mechanical stimuli seemed to have been indirect, perhaps via the release of chemical mediators by the mechanical stimulus, because the excitation reproducibly required strong and extended prior probing of the receptive field, and the discharge elicited outlasted the cessation of the stimulus by many minutes.

The ic RX 77368-evoked splanchnic afferent response typically lasted 40–60 min. This duration exceeds by one to two orders of magnitude that observed in response to peripherally (topically or ia) applied chemical excitants (e.g., bradykinin, serotonin, lactic acid, or substance P) in feline splanchnic units innervating various abdominal organs including stomach (Lew and Longhurst 1986; Longhurst et al. 1984; Pan et al. 1994; Stahl and Longhurst 1992). In contrast, the time course of splanchnic afferent activation after ic RX 77368 is consistent with, although somewhat shorter than, the prolonged course of ic RX 77368-evoked vagal afferent activation driving functional changes in the stomach (Kiraly et al. 1998; O-Lee et al. 1997; Thieflin et al. 1989). Intracisternal RX 77368 or TRH, at or above the doses used in these experiments, cause vagal afferent excitation beginning ±5 min postinjection and lasting ~45 (TRH, 3 ng) or 90 (RX 77368, 1.5 ng) min (O-Lee et al. 1997). Functional studies have shown that ic RX 77368
enhances GMBF through vagal muscarinic pathways (Kiraly et al. 1998; Thießen et al. 1989) and that this gastric mucosal hyperemia depends on the release of CGRP from capsaicin-sensitive extrinsic afferent fibers (Kiraly et al. 1997). These results provide electrophysiological evidence that the afferent signaling function of splanchnic fibers is also recruited by activation of central TRH pathways. Gastric mucosal vasodilation and splanchnic afferent signaling in this model are likely to occur concomitantly. Preliminary measurements of GMBF with laser Doppler flowmetry in urethane-anesthetized rats indicate that the onset and latency to peak of RX 77368-induced gastric hyperemia are similar to those observed for splanchnic afferent excitation (K. Kawakubo and Y. Tache, unpublished observations). The gastric splanchnic units responsive to RX 77368 are likely to include CGRP-containing fibers because ~90% of splanchnic afferent fibers in the rat are CGRP immunopositive (Kashiba et al. 1991; Sternini and Anderson 1992), and they may comprise some or all of those whose efferent function (vasodilation of the splanchnic vasculature via CGRP release) is elicited as a result of activation of TRH-sensitive neurons in the brain stem (Kiraly et al. 1997; Raggenbass et al. 1990).

The mechanisms responsible for exciting splanchnic afferent units after ic RX 77368 administration are not known, although a number of changes in gastric physiology resulting from this stimulus have been characterized. In urethane-anesthetized animals, low ic doses of RX 77368 cause a sustained increase in tonic intragastric pressure of between 10 and 20 cm H$_2$O beginning ~5 min after ic injection, over which is superimposed ongoing rhythmic contractions with amplitudes of several cm of H$_2$O (T. Nozu and Y. Tache, unpublished observations). In the current work, RX 77368-provoked splanchnic afferent discharge was, in most cases, neither rhythmic nor episodic, suggesting that gastric contractions per se were unlikely to be the immediate source of the excitation. However, in several units with high initial ongoing discharge rates, rhythmic or episodic bursts of activity did occur after an unpatterned increase in mean discharge rate. In the two units with clear rhythmic discharge, the rhythmic activity had a period of ~45 s to 1 min, similar to the periodicity observed in recordings of intragastric pressure under similar conditions (T. Nozu and Y. Tache, unpublished observations) and was observed for ~10 min during an interval surrounding the period of peak discharge (e.g., Fig. 4C). It is possible that the brief period of rhythmic discharge may have resulted from a combined effect of gastric contractions and chemical excitants but that mechanical modulation of activity only occurred during the period of greatest chemical excitation. A dual modulation via chemical and mechanical stimuli would be consistent with the polymodal responsiveness of many visceral afferent neurons including those in the splanchnic nerve (Adelson et al. 1997a;
FIG. 4. Patterns of discharge in response to 3 ng ic RX 77368. In each panel the period of the discharge shown in the large panel (instantaneous frequency plot) is the period between the hatched vertical lines in the inset histograms. The arrow in each histogram indicates the time at which RX 77368 was injected ic. A, B, and C were recorded simultaneously from the same nerve twig and the intervals illustrated for each pair coincide. A: most units tested responded in the manner shown in this panel, with increasing irregular discharge. B: in this unit, several bursts interrupted otherwise irregular activity. No similar change in activity was observed during these bursts in the unit shown in A. C: discharge in this unit developed a discernible rhythmicity ~15 min after RX 77368 injection that lasted ~10 min.

JaÈ nig and Morrison 1986; Kumazawa 1986; Kumazawa et al. 1988; Lew and Longhurst 1986; Longhurst and Dittman 1987). Because different units present in a single filament could display quite different patterns of discharge simultaneously (Fig. 4), local events in the terminal microenvironment may play an important role in the modulation of discharge. Alternatively, these differences may indicate a diversity of unit types.

Gastric acid secretion has been previously measured at the doses of RX 77368 used in the current experiments. It is not significantly increased above basal levels at 1.5 ng in urethan-anesthetized animals (Kiraly et al. 1997), although it is just significantly elevated at the 3-ng dose (Raybould et al. 1990). That a majority of units with ongoing activity responded after ic RX 77368 at either dose suggests that reductions in luminal pH are unlikely to be the immediate cause of the observed changes in afferent discharge. Additional evidence arguing against a role for luminal acid secretion in splanchnic afferent excitation in these experiments is the preliminary finding that indomethacin pretreatment to inhibit gastric prostaglandin release (Yoneda and Tache 1993) reduces RX 77368-evoked splanchnic afferent discharge (Adelson et al. 1997b), while it increases gastric acid secretion in response to either acid secretory or normally nonsecretory ic doses of RX 77368 (Kiraly et al. 1997; Yoneda and Tache 1993). Similarly, the CGRP-dependent gastric hyperemia after ic TRH analogue (Kiraly et al. 1994, 1997) is not altered by omeprazole-induced suppression of acid secretion (Tanaka et al. 1997; Thiefin et al. 1989).

Other possible chemical excitants of gastric splanchnic afferent fibers released after ic RX 77368 injection may include serotonin and/or histamine. Although the amounts of histamine and serotonin released by the ic doses of RX 77368 used in these experiments have not been measured, higher ic doses of RX 77368 are known to cause significant increases in gastric luminal secretion of these mediators (Stephens and Tache 1989; Yanagisawa and Tache 1990; Yang et al. 1992).

Regardless of the identity of the immediate excitant(s) of splanchnic afferent terminals resulting from low ic doses
of TRH analogue, it is of interest to assess whether the stimulus may be considered noxious, given the prominence of nociception among the recognized afferent functions of the splanchnic nerve (Cervero 1994). As described above, low doses of ic RX 77368 in urethan-anesthetized rats cause at most small increases in gastric acid secretion and moderate changes in gastric motility, although they elicit substantial increases in GMBF in urethan-anesthetized rats (Kato et al. 1996; Kiraly et al. 1997; Raybould et al. 1990). Intracisternal doses of RX 77368 several times greater than those used in this study protect the gastric mucosa against damage by challenge with noxious agents such as ethanol via a combination of increased GMBF and prostaglandin synthesis (Kato et al. 1995, 1996). One approach to investigating visceral nociception has been to define noxious stimulus intensities as those capable of evoking pseudoaffective autonomic reflex responses (Cervero 1982). Low ic doses of RX 77368 do cause significant increases in mean arterial pressure (~15 mmHg) in urethan-anesthetized rats (Kiraly et al. 1997). However, this effect is unaltered in animals pretreated with capsaicin, suggesting that it results from changes in sympathetic efferent activity evoked directly by ic RX 77368 rather than from reflexes driven by peripheral afferent excitation (Kiraly et al. 1997). It therefore seems unlikely that the splanchnic afferent excitation resulting from central autonomic activation by low ic doses of TRH analogue should be considered nociceptive.

The ic TRH-evoked changes in gastric function provide a useful system in which to study the physiological role of splanchnic afferent units. The highest concentration of medullary TRH receptors is found in the medial DMN, where the cell bodies of preganglionic vagal neurons modulating gastric function are localized (Tache et al. 1993), and this region receives dense projections of TRH-immunopositive fibers from the medullary raphe nuclei (Lynn et al. 1991). These pathways appear to be involved in the vagally mediated gastric responses to cold restraint or sham feeding in conscious animals (Barrachina et al. 1997; Martinez et al. 1998). Thus, although the impulse activity elicited in splanchnic afferent fibers after ic RX 77368 may not be nociceptive, it is likely to be involved in patterns of autonomic activation and regulation yielding physiologically adaptive responses to a variety of stimuli.

The possible physiological functions of the splanchnic afferent discharge evoked by low doses of ic RX 77368 may include the modulation, via spinal reflex arcs and/or peripheral reflex arcs through sympathetic ganglia, of sympathetic drive to enteric ganglia or to blood vessels, which in turn may influence gastrointestinal motility, secretion, or vascular tone (Furness 1991). In addition, these signals may also influence activity of medullary neurons via projections to the DVC (Renehan et al. 1995).

Part of the slow progress in determining visceral afferent function is that we have much less familiarity with the stimuli relevant to the gut than we do with stimuli relevant to, for example, the skin. Centrally administered TRH analogue elicits physiologically relevant activation of autonomic nervous activity that evokes nonnoxious (at the doses used in the current experiments) changes in gastric function, including the release of CGRP from splanchnic afferent nerve terminals (Kiraly et al. 1997), and an increase in splanchnic afferent discharge. Thus in this model the dual afferent/efferent function of small-diameter dorsal root ganglion neurons is recruited by centrally mediated changes in autonomic function.
nervous activity. This “systems” stimulus provides a useful tool to investigate the role of the splanchic afferent nerve supply in physiologically relevant autonomic coordination of gastrointestinal function.

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