Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats

GERARD P. SMITH, CYNTHIA JEROME, AND RALPH NORGREN
Eating Disorders Institute, Department of Psychiatry, Cornell University Medical College and
New York Hospital-Cornell Medical Center, Westchester Division, White Plains, New York 10605;
and Department of Behavioral Science, College of Medicine, Pennsylvania State University,
Hershey, Pennsylvania 17033

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GREN. Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. Am. J. Physiol. 249 (Regulatory
Integrative Comp. Physiol. 18): R638-R641, 1985.—Selective
section of afferent vagal axons that reach below the diaphragm
blocks the satiating effect of peripherally administered chole-
cystokinin in the rat. Section of the analogous efferent axons
has no effect. After the behavioral tests, the selective axonal
sections were confirmed with horseradish peroxidase histo-
chemistry.

vagal axons; food intake

SMITH ET AL. (12) reported in 1981 that total abdominal
vagotomy or selective gastric vagotomy abolished or
markedly reduced the satiating effect of peripherally
administered cholecystokinin (CCK-8). This effect of
total vagotomy was confirmed by Lorenz and Goldman
(5) and by Morley et al. (6). Because the abdominal vagal
nerves contain afferent and efferent fibers, it is unclear
whether the critical lesion involved afferent or efferent
fibers. When peripheral anticholinergic blockade by atro-
pine methyl nitrate, which provides a crude pharmacolog-
ic mimic of a lesion of efferent vagal fibers, did not
block the satiating effect of CCK-8, Smith et al. (12)
proposed that the critical lesion involved afferent vagal
fibers. By using a new surgical technique that permits
selective sectioning of afferent or efferent vagal fibers,
we can report evidence that confirms the afferent fiber
hypothesis. A preliminary report of these results has
appeared (13).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Charles River, Cambridge,
MA) weighing 220–450 g at the time of rootlet surgery
were housed individually and tested in hanging wire cages
in a room with lights on from 0700 to 1900 that was
maintained at 22 ± 2°C. Rats were maintained on a sweet
milk diet (Magnolia, Borden Foods, Columbus, OH) di-
luted 50% (vol/vol) with tap water containing Johnson’s
Baby Vitamins, 0.2% by volume. The maintenance and
test diets and tap water were presented in graduated
drinking tubes (Wahmann Manufacturing, Timonium, MD).
Tap water was available ad libitum.

Surgery

Vagal afferent and efferent rootlet surgery. We modified
the ventral surgical approach to the medulla described
by Travers and Norgren (15). This modification permits
microscopic visualization of the afferent and efferent
vagal rootlets as they approach the lateral surface of the
medulla. Selective afferent or efferent rootlet section is
possible because, in the rat, the afferent rootlets enter
the medulla dorsal to the site where efferent rootlets exit
(2–4). After pretreatment with atropine (0.1 mg) and
anesthetization with pentobarbital sodium (Nembutal,
50 mg/kg), the rats were placed supine in a head holder.
With the aid of an operating microscope, the basal aspect
of the occipital bone was exposed by blunt dissection
after tying off and sectioning the superior thyroid artery
and removing the posterior wing of the hyoid bone with
its attached musculature. The posterior lacerated fora-
men was expanded medially by thinning the occipital
bone with a dental burr and then removing the remaining
bone posteriorly to the level of the hypoglossal canal
with fine forceps or a modified corneoscleral punch. This
exposure revealed the vagus nerve as it penetrates
through the dura mater and separates into two groups of
nerve rootlets. The rootlets in the ventral group are
smaller and more numerous, and many extend caudally
before penetrating the ventrolateral medulla. The dorsal
group consists of two to four rootlets that reach the
dorsalateral surface of the medulla at the level of the
posterior lacerated foramen. Once the dura is lanced, the
ventral (afferent) rootlets can be sectioned with iris
scissors. With this ventral approach the dorsal rootlets
often are partially obscured by the overlying ventral

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rootlets and accompanying veins. These must be deflected gently before the dorsal rootlets can be sectioned. In addition to the vagus, efferent section involves both the accessory (XI) and glossopharyngeal (IX) roots. Likewise, an afferent transection includes both vagal and glossopharyngeal axons, because in the rat these two cranial nerves join in a common sheath at the level of the posterior lacerated foramen. All rats underwent rootlet section on the left side.

Abdominal vagotomy. About 2 wk after the rootlet operation, unilateral abdominal vagotomy was performed to lesion the afferent and efferent fibers connected contralateral to the side of the rootlet section (Fig. 1). Due to the partial rotation of the stomach during development, the left (or posterior) subdiaphragmatic vagus is continuous with the right cervical vagus nerve and connects to the right side of the medulla. Thus the unilateral abdominal vagotomy was conducted on the left trunk according to the procedure described by Smith and Jerome (11). Briefly, the left trunk was identified just below the diaphragm. Two 3-0 silk sutures were tied around the vagal trunk, 1-2 cm apart, and the nerve between the sutures was sectioned. The sutures restricted regeneration of transected fibers and facilitated anatomic verification of vagotomy at the conclusion of the experiment.

The rationale for this combination of intracranial rootlet and abdominal vagotomy lesions is twofold. First, anterograde and retrograde transport of horseradish peroxidase (HRP) from abdominal vagal nerves shows no significant exchange of fibers between the right and left vagal nerves from the level of the medulla to below the diaphragm (1, Norgren and Smith, unpublished observations) (Fig. 1). Second, unilateral abdominal vagotomy does not reduce the satiating potency of peripherally administered CCK-8 (10).

Food Intake Tests

About 2 wk after unilateral abdominal vagotomy rats were adapted to a 17-h deprivation schedule, and the intake of a high-carbohydrate liquid diet (Bio Serv EC116 liquid diet, Bio Serv, Frenchtown, NJ) was measured 15 and 30 min after intraperitoneal administration of CCK-8 (6 μg/kg body wt) or isovolumetric 0.15 M saline. Under these conditions the 30-min intake is the size of the meal.

The CCK-8 (a gift from Squibb, Princeton, NJ) was dissolved in 0.15 M saline just before each test. This dose was chosen because in other experiments it produced a nearly maximal inhibition of food intake without a significant inhibition of water intake (9).

Lesion Verification

Afferent and efferent vagal rootlets. At the conclusion of the behavioral experiments we anesthetized each rat with serial injections of atropine sulfate (0.1 mg ip), pentobarbital sodium (30 mg/kg ip), and ethyl carbamate (urethan, 0.7 g/kg ip) spaced over 30 min. The central end of the cut cervical vagal trunk ipsilateral to the rootlet lesion was then exposed to crystalline horseradish peroxidase (HRP, Boehringer Mannheim grade I) using a protocol described in detail by Hamilton and Norgren (3). The vagal trunk was always exposed to HRP crystals for 6 h.

After a 60- to 70-h survival the rats were reanesthetized with pentobarbital sodium (100 mg/kg ip) and then perfused via the left ventricle with 0.9% saline (4-5 min), 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3, 20 min), and cold 5% sucrose in 0.1 M phosphate buffer (20 min). The caudal brain stem and upper spinal cord were removed immediately, cut coronally at 40 μm on a freezing microtome, and reacted as described by Hamil-

FIG. 1. Surgical preparation required to selectively section afferent or efferent axons in abdominal vagus nerves. Dashed lines, course of afferent axons; lines rising from triangles, course of efferent axons. A: combining section of afferent rootlets of 1 vagus nerve with complete section of subdiaphragmatic trunk that connects to contralateral medulla prevents abdominal vagal afferent activity from reaching brain, depicted by a schematic coronal section through caudal medulla, but permits efferent activity to reach the brain via 1 vagal trunk. B: a similar preparation in which efferent rather than afferent rootlets are severed in 1 nerve prevents all vagal efferent axons from reaching the gut but permits abdominal afferent activity to reach the brain via 1 vagal trunk. ap, Area postrema; mX, dorsal motor nucleus of the vagus; mXII, hypoglossal nucleus; na, nucleus ambiguus; nst, nucleus of solitary tract; ST, solitary tract.
FIG. 2. Dark-field photomicrographs of coronal sections through nucleus of solitary tract and dorsal motor nucleus of vagus at level of area postrema. Central end of cut cervical vagus nerve was exposed to horseradish peroxidase (HRP) before medulla was sectioned and stained for anterograde and retrograde transport of peroxidase, HRP reaction product after afferent vagal rootlet section (A), full surgical control procedure (B), and efferent vagal rootlet section (C). See Fig. 1 for abbreviations.

TABLE 1. Effect of selective bilateral afferent or efferent section of abdominal vagal fibers on CCK-induced satiety

<table>
<thead>
<tr>
<th>Intake, ml/30 min</th>
<th>%Inhibition</th>
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<tbody>
<tr>
<td><strong>Saline</strong></td>
<td><strong>CCK</strong></td>
</tr>
<tr>
<td>1 19 19</td>
<td>0</td>
</tr>
<tr>
<td>2 14 16</td>
<td>-14</td>
</tr>
<tr>
<td>3 12 16</td>
<td>-33</td>
</tr>
<tr>
<td>4 14 17</td>
<td>-21</td>
</tr>
<tr>
<td>5 16 15</td>
<td>5</td>
</tr>
<tr>
<td>6 15 13</td>
<td>13</td>
</tr>
<tr>
<td><strong>Afferent lesion</strong></td>
<td><strong>Efferent lesion</strong></td>
</tr>
<tr>
<td>1 12 7</td>
<td>44</td>
</tr>
<tr>
<td>2 12 6</td>
<td>50</td>
</tr>
<tr>
<td>1 12 6</td>
<td>50</td>
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Percentage of inhibition of food intake = 100 × [1 - 30 min intake after CCK-8/30 min intake after saline]. Negative inhibition indicates more was eaten after cholecystokinin (CCK) than after saline. Afferent lesion data are summarized as means ± SE. Control animal was a full surgical control.

RESULTS AND DISCUSSION

Nine of 15 rats that underwent rootlet surgery had verified rootlet and vagotomy lesions: six had afferent rootlet lesions, two had efferent rootlet lesions, and one was a full surgical control. The marked difference in the pattern of HRP reaction product within the nucleus of the solitary tract and dorsal motor nucleus of the vagus after afferent or efferent rootlet section is illustrated in Fig. 2.

The effect of CCK-8 on the food intake of these rats is summarized in Table 1. The combination of a unilateral afferent rootlet lesion with section of the abdominal vagal trunk containing the afferent and efferent fibers connected to the contralateral medulla (Fig. 1A) abolished the satiating effect of CCK-8. However, the combination of a unilateral efferent rootlet lesion with section of the abdominal vagal trunk containing the afferent and efferent fibers connected to the contralateral medulla (Fig. 1B) did not appear to alter the satiating effect of CCK-8 from that observed in the full surgical control (Table 1).

The differential effect of afferent and efferent rootlet lesions in these experiments confirms the hypothesis that section of afferent fibers is the critical lesion for blocking the satiating effect of peripherally administered CCK-8. The site of activation of these fibers by circulating CCK-8 is unknown. The most likely possibilities are that CCK-8 activates the vagal afferent fibers either indirectly through a smooth muscle contractile effect in the pyloric sphincter region (14) or directly through CCK-8 receptors on the axons themselves (16).

In addition to its specific application to the mediation of the satiating effect of peripherally administered CCK-8, we emphasize the usefulness of the afferent or efferent rootlet lesion procedure for analyzing the relative contribution of afferent or efferent abdominal vagal fibers to the effect of vagotomy on other aspects of eating (8) and drinking behavior (11), on gastrointestinal secretion and motility, and on neuroendocrine and metabolic functions of the abdominal viscera.

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