Signaling the brain in systemic inflammation: which vagal branch is involved in fever genesis?

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Simons, Christopher T., Vladimir A. Kulchitsky, Naotoshi Sugimoto, Louis D. Homer, Miklos Székely, and Andrej A. Romanovsky. Signaling the brain in systemic inflammation: which vagal branch is involved in fever genesis? *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R63–R68, 1998.—Recent evidence has suggested a role of abdominal vagal afferents in the pathogenesis of the febrile response. The abdominal vagus consists of five main branches (viz., the anterior and posterior celiac branches, anterior and posterior gastric branches, and hepatic branch). The branch responsible for transducing a pyrogenic signal from the periphery to the brain has not as yet been identified. In the present study, we address this issue by testing the febrile responsiveness of male Wistar rats subjected to one of four selective vagotomies: celiac (CBV), gastric (GBV), hepatic (HBV), or sham (SV). In the case of CBV, GBV, and HBV, only the particular vagal branch(es) was cut; for SV, all branches were left intact. After the postsurgical recovery (26–29 days), the rats had a catheter implanted into the jugular vein. On *days 29–32*, their colonic temperature (T_c) responses to a low dose (1 μg/kg) of *Escherichia coli* lipopolysaccharide (LPS) were studied. Three days later, the animals were subjected to a 24-h food and water deprivation, and the effectiveness of the four vagotomies to induce gastric food retention, pancreatic hypertrophy, and impairment of the portorenal osmotic reflex was assessed by weighing the stomach and pancreas and measuring the specific gravity of bladder urine, respectively. Stomach mass, pancreas mass, and urine density successfully separated the four experimental groups into four distinct clusters, thus confirming that each type of vagotomy had a different effect on the indexes measured. The T_c responses of SV, CBV, and GBV rats to LPS did not differ and were characterized by a latency of ~40 min and a maximal rise of 0.7 ± 0.1, 0.6 ± 0.1, and 0.9 ± 0.2°C, respectively. The fever response of the HBV rats was different; practically no T_c rise occurred (0.1 ± 0.2°C). The HBV appeared to be the only selective abdominal vagotomy affecting the febrile responsiveness. We conclude, therefore, that the hepatic vagus plays an important role in the transduction of a pyrogenic signal from the periphery to the brain.

temperature regulation; neuroimmunomodulation; febrile response; lipopolysaccharides; liver; selective vagotomy; rats

AFTER THE INVASION of pathogens, the host organism recruits nonspecific defense mechanisms in a dynamic, time-dependent response, the sickness syndrome (23), which consists of early symptoms (such as hyperalgesia/allodynia, motor agitation, and fever) followed by late symptoms (including hypoalgesia, motor depression, and either fever or hypothermia). The sickness syndrome develops under the control of the central nervous system, but it is not clear how the brain receives information about the pathogenic invasion in the periphery. Several concepts have been proposed (for review, see Refs. 7, 34), and one of them, neural route signaling, is detailed in this paper.

This concept suggests that the vagus nerve serves as an informational highway for inflammatory signals originating outside the brain. It has been demonstrated that animals with bilateral truncal subdiaphragmatic vagotomy respond to a peripheral injection of a bacterial lipopolysaccharide (LPS) or a pyrogenic cytokine [such as interleukin-1 (IL-1)] with an abated sickness syndrome; several symptoms, including hyperalgesia (36, 36), sleep (16), and the depression of social investigation (8, 9), either do not occur or are attenuated. The fever response, the focus of this work, is also suppressed in vagotomized animals, as it has been shown in rats and guinea pigs, by using both LPS and IL-1 (15, 16, 24, 26, 28, 33). Moreover, in the case of fever, it has been clarified that the decreased responsiveness of vagotomized animals is indeed because of missing vagal afferentation and is due neither to the absent vagal efferentation nor to any severe complications (side effects) of vagotomy such as malnutrition. Thus Székely et al. (31) have shown that LPS fever is depressed if chemosensitive intra-abdominal neural afferents (presumably, including vagal; the vagus is a major source of visceral innervation) are desensitized with intraperitoneal capsaicin. In another group of studies, Romanovsky and co-workers (24, 25, 30) proposed that the febrile nonresponsiveness of vagotomized rats results from the defective transduction of pyrogenic signals to the brain. The authors did so by excluding potential alternatives, viz., malnutrition-dependent and -independent thermoeffector incompetence (Refs. 24 and 25, respectively) and vagotomy-associated activation of endogenous antipyresis (30).

Knowing that afferent fibers travel within all branches of the abdominal vagus (anterior celiac, posterior celiac, anterior gastric, posterior gastric, and hepatic; see Fig. 1), it is logical to ask the following question: The interruption of which branch is actually responsible for fever suppression in animals with subdiaphragmatic vagotomy? Rats were subjected to one of four selective vagotomies (celiac, gastric, hepatic, or sham), and their febrile responsiveness to a low dose of LPS (1 μg/kg iv) was tested. In our previous studies (24, 26), we have shown that the febrile response to this dose of LPS is prevented by truncal subdiaphragmatic vagotomy.

METHODS

Animals. Thirty-six adult male Wistar rats (B & K Universal; Kent, WA) weighing ~350 g were used in the experi-

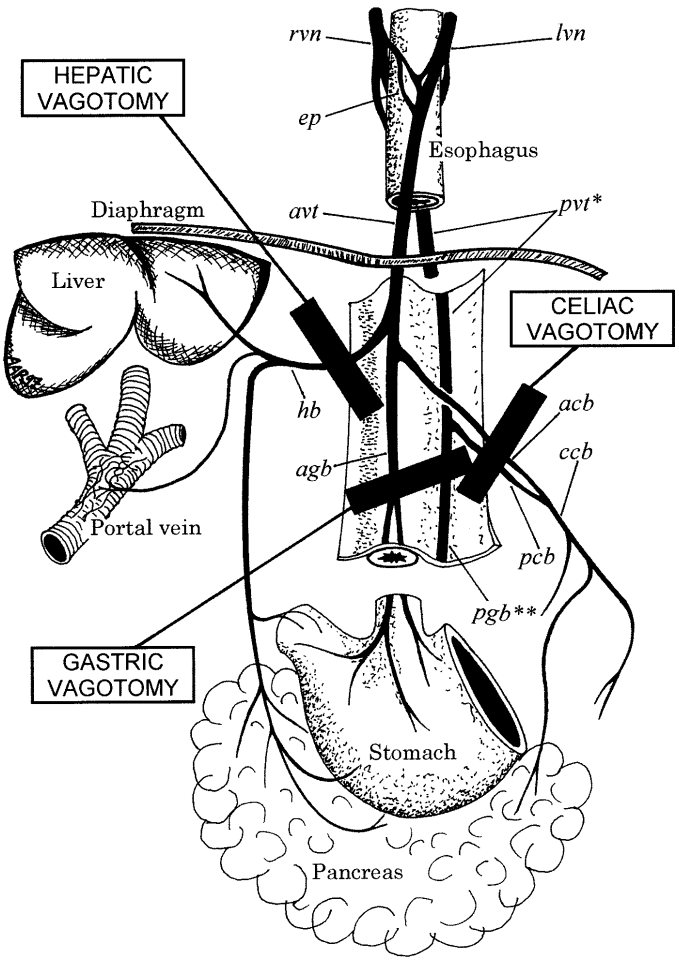

Fig. 1. Three types of selective subdiaphragmatic vagotomy, viz., celiac, gastric, and hepatic, are shown on a schematic representation of typical distribution of rat abdominal vagal branches. acb, Anterior celiac branch; agb, anterior gastric branch; avt, anterior vagal trunk; ccb, common celiac branch; ep, esophageal plexus; hb, hepatic branch; lvn, left vagus nerve; pcb, posterior celiac branch; pgb, posterior gastric branch; pvt, posterior vagal trunk; rvn, right vagus nerve. For sake of clarity, 2 simplifications are made. First (*), dorsal esophageal surface is shown as if serosa was cut along right side of esophagus, partially separated from its underlying tissues, and laid in a frontal plane; posterior vagal trunk was transected immediately below diaphragm. Second (**), posterior gastric branch is shown only to level of lesser gastric curvature, with distal portion being entirely omitted.

ments. The animals were initially housed three per cage; for the last 4–5 days before the experiment, they were housed singly. The room was maintained on a 12:12-h light-dark cycle (lights on at 7 AM); ambient temperature (T_a) was kept at 22 ± 1°C. Food and water were available ad libitum; the vagotomized animals were given reinforced food (24). The animals were handled regularly; during the last 10 days before the experiments, they were also habituated (5 training sessions of 3–4 h each) to a cylindrical, wire restrainer that lightly restricted their back and forth movements. These same restrainers were used later in the experiments. To obviate any possible effects of circadian rhythms, the experiments were always started between 9 and 10 AM.

Surgical preparation. To lessen the severity of surgery, the animals were operated on in two stages. First, 1 wk after their acquisition, the rats were food deprived for 12 h and thereafter anesthetized with an intraperitoneal injection of ketamine-xylazine-acepromazine cocktail (55.6, 5.5, and 1.1 mg/kg, respectively). Immediately thereafter, they were given a prophylactic injection of antibiotic (enrofloxacin, 12 mg/kg sc). The animals were randomly assigned to four groups and underwent one of four vagotomies: celiac branch vagotomy (CBV), gastric branch vagotomy (GBV), hepatic branch vagotomy (HBV), or sham vagotomy (SV). In all cases, a midline laparotomy was performed, and the stomach and lower esophagus were retracted from the abdominal cavity and covered with sterile, saline-moistened gauze. For CBV, the posterior and anterior celiac branches were isolated and cut, each ~5 mm distal to its confluence with the corresponding vagal trunk (Fig. 1). Similarly, for GBV, the anterior and posterior gastric branches were individually cut 10 mm from their junctions with the trunks (Fig. 1). For HBV, the liver was deflected to expose the hepatic vagal branch, which was then isolated and transected 5 mm from the anterior vagal trunk (Fig. 1). In SV, the viscera were similarly retracted from the peritoneal cavity to expose the trunks, but no branches were cut. Thereafter, another dose of enrofloxacin (12 mg/kg ip) was administered, and the laparotomy wound was sutured in two layers. The animal was then transferred to a stereotaxic apparatus, and a small acrylic platform was affixed to the skull with four miniature screws; this platform was used later to accommodate a hollow pedestal protecting the free end of an intravenous catheter.

After a 26- to 29-day recovery period, stage two of the surgical preparation was performed. Under anesthesia and preoperative antibiotic protection (same as above), the right jugular vein was exposed through a small skin incision and ligated. Thereafter, a Silastic catheter (0.9 mm OD) filled with heparinized (100 U/ml) pyrogen-free saline (PFS) was inserted through the jugular vein into the superior cava vein and secured with surgical thread. The free end of the catheter was tunneled under the skin, exteriorized at the base of the skull, coiled, and placed into a hollow pedestal. The pedestal was affixed to the acrylic platform (using dental acrylic) and protected with a screw-on cap. Postsurgically, the catheter was flushed with heparinized PFS every other day, and its patency was verified by the presence of blood and low resistance.

Experimental protocol. The febrile responsiveness to intravenous LPS was tested 29–32 days after the selective vagotomy. On the day of the experiment, each animal was placed into its restrainer and instrumented with a colonic thermocouple (inserted to 9 cm beyond the anus). The thermocouple was connected to a data acquisitor (model TCA-A1–24, Dianachart, Rockaway, NJ) and then to a computer. The rat was then placed into an environmental chamber (Forma Scientific, Marietta, OH) set to an T_a of 30.0°C (upper limit of thermoneutrality for rats) and a relative humidity of 50%. The exteriorized portion of the intravenous catheter was pulled through a wall port and connected to a syringe. After a 1-h stabilization period, measurements were begun, and colonic temperature (T_c) was recorded every 2 min for 5 h. In one-half of the experiments, the animals received the intravenous injection of *Escherichia coli* 0111:B4 LPS (1 μg/kg; Sigma, St. Louis, MO) in PFS (1 ml/kg) 1 h after the beginning of measurements. In the other half, no injection was performed (controls). This paradigm for the control experiments was chosen because the injection of PFS per se was already shown to have no substantial effect on T_c (26), yet the circadian rhythm of T_c and potential effects of the relatively long-lasting restraint needed to be evaluated. Each rat was tested twice. One-half of the animals received LPS during the first test and, 3 days later, were used in control experiments;

the other half was studied in a counterbalanced order, i.e., control followed by the LPS test 3 days later.

Effectiveness of vagotomy. The effectiveness of each type of selective vagotomy was verified at the end of the study. Three days after the last experiment, each animal was deprived of food and water for 24 h, and its bladder urine was collected (37) and sent to a clinical laboratory for refractometric determination of the specific gravity (density). Thereafter, the animal was killed with pentobarbital sodium (50 mg/kg iv), and its stomach and pancreas were carefully isolated, removed, and weighed. The effectiveness of the selective vagotomies was verified based on three criteria: *1*) the stomach (together with its content) mass (at the end of a 24-h food deprivation), *2*) the pancreas mass, and *3*) the specific gravity of the urine (at the end of a 24-h water deprivation). The first criterion assesses the evacuatory function of the stomach. The transection of the gastric branches (GBV) is known to cause paralysis of gastric musculature and the resultant retention of food in the stomach (13, 18). The transection of other vagal branches is unlikely to affect the stomach mass to the same extent, because the efferent innervation of the stomach is accomplished mostly by the two gastric branches (6). The second criterion allows for revealing pancreatic hypertrophy, which develops in animals with various types of vagotomy, e.g., highly selective gastric vagotomy (27). Because the efferent innervation of the pancreas and surrounding jejunum comes from all major vagal branches (6), it could be expected that CBV, GBV, and HBV would all affect the pancreas mass. [It could also be expected that the transection of the hepatic branch would have the smallest effect, because this branch contains the smallest absolute number of fibers and the lowest proportion of efferent fibers (22).] The third criterion assesses the effectiveness of the portorenal osmotic reflex (urine concentration after water deprivation), which has been suggested to originate from portal osmoreceptors (hepatic branch) and shown to be affected by HBV (1).

Data processing and analysis. To evaluate the thermal response, we used the change in T_c (ΔT_c) and the fever index. The former was calculated as a deviation from the T_c at *time 0* (i.e., the time of the injection in the LPS experiments and 1 h after the beginning of recording in the controls); the latter was calculated by integrating each ΔT_c curve over 0–4 h. The stomach and pancreas masses were expressed relative to the body mass. To verify the effectiveness of selective vagotomies, a randomization-based analysis of clusters was performed by calculating intergroup distances in the three-dimensional (relative stomach mass, relative pancreatic mass, and urine density) space. In this study, we also applied one-way and two-way ANOVA and Dunnett's post hoc test, as appropriate. All data are presented as means ± SE.

RESULTS

Thermal responses. The animals of the four groups studied (SV, CBV, GBV, and HBV) were all in good condition. At the time of the experiment, their body mass was between 324 ± 13 g (SV) and 346 ± 18 g (HBV), and the initial T_c ranged from 37.9 ± 0.1°C (GBV) to 38.1 ± 0.1°C (SV and CBV). In the control experiments, all groups exhibited similar steady T_c declines ($P = 0.84$; Fig. 2). To the dose of LPS used (1 μg/kg iv), the sham-vagotomized rats (Fig. 2*A*) responded with low-grade fevers (some appearing monophasic and others biphasic); the mean ΔT_c curve was characterized by a ~40-min latency and a peak of 0.7 ± 0.1°C occurring at 130 min postinjection. The febrile responses of the animals with celiac (Fig. 2*B*) or gastric (Fig. 2*C*) vagotomy were similar to the responses of the sham group ($P = 0.45$, Dunnett's test); the mean ΔT_c reached 0.6 ± 0.1 in the CBV and 0.9 ± 0.2°C in the GBV group. In contrast, the rats with hepatic vagotomy (Fig. 2*D*) responded to the LPS with a strongly suppressed fever (0.1 ± 0.2°C) as compared with the shams ($P = 0.02$). The intergroup comparison of the 4-h fever index led to the same conclusion: the response of the HBV group was significantly ($P = 0.02$) different from those of the SV, CBV, and GBV groups (Fig. 3).

Effectiveness of vagotomy. Food retention in the stomach (assessed by the relative stomach mass), pancreatic hypertrophy (assessed by the relative mass of the pancreas), and inefficiency of the portorenal osmotic reflex (assessed by the specific gravity of urine) separated the four experimental groups into four distinct clusters (Fig. 4), thus confirming that each type of vagotomy had its own effect on the set of indexes measured ($P < 0.02$, randomization test). Although each of the indexes did contribute significantly to the intercluster separation, none of them, taken alone, allowed for differentiating between all four groups, probably due to the substantial anatomical overlap between the basins of different abdominal vagal branches and their partial functional redundancy (6, 18). Unexpectedly, the fever index, by itself, successfully separated the SV and HBV groups (Fig. 4), whereas the specifically selected test, urine density, did not. However, when the fever index (instead of the urine density) was used in the cluster analysis, this did not further improve the intercluster separation.

DISCUSSION

Our data show that the integrity of the hepatic vagal branch, but not the celiac or gastric branches, is necessary for the development of the fever response to a low dose of intravenous LPS. This suggests that some important mechanisms of pyrogen processing are localized in the liver. The involvement of this organ in the pathogenesis of fever has been suspected for a long time (12) and is supported by a variety of findings, of which the hepatic clearance of peripherally injected pyrogens is well documented (10, 20). In addition, it has been demonstrated (4) that infusion of LPS into the portal vein of guinea pigs increases the metabolic rate more readily than either an intermittent or continuous infusion of LPS into the peritoneal cavity. Immunologic correlates of such route dependence have been obtained in studies by Marshall et al. (19) and by Battisto and Miller (5). In the former study, the intraportal administration of live *E. coli* resulted in a greater suppression of the delayed-type hypersensitivity responses than did systemic administration; in the latter work, the introduction of either complete antigens or haptens into portal tributaries resulted in the induction of specific tolerance, whereas a systemic administration of the same materials did not. Our recent observations in rats with congested liver also agree with the involvement of this organ in fever mechanisms; the hepatomegalic rats responded to a biphasic fever-inducing dose of intrave-

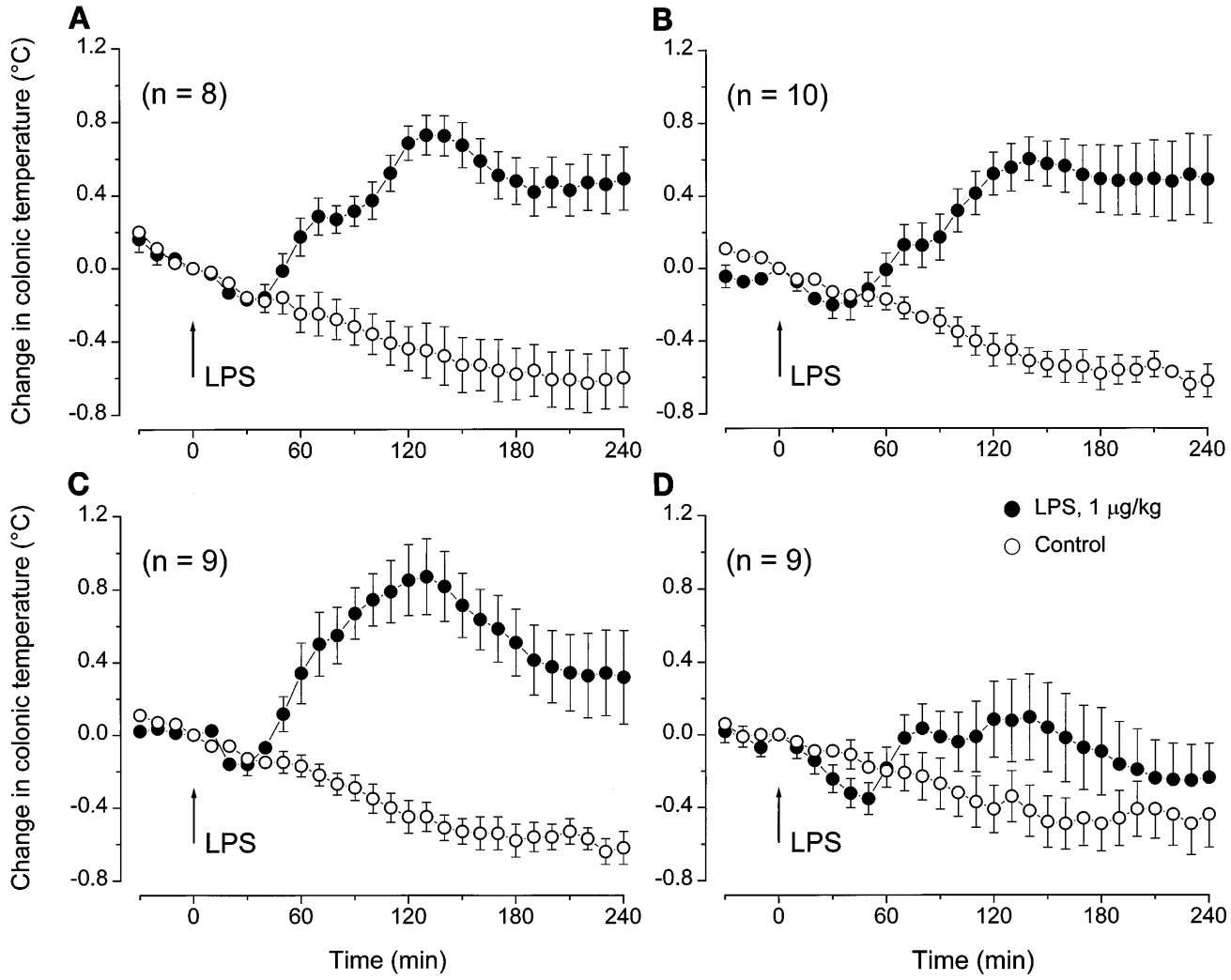

Fig. 2. Dynamics of colonic temperature in lipopolysaccharide (LPS)-treated vs. nontreated rats subjected to 4 different types of selective subdiaphragmatic vagotomy: sham (*A*), celiac (*B*), gastric (*C*), and hepatic (*D*). LPS (1 μg/kg iv) was injected (arrow) at *time 0*.

nous LPS with an attenuated first phase (32). As for the immunologic responsiveness, it has been known for a long time to be altered in liver pathology. Thus, already 20 years ago, Cantor and Dumont (11) found that portocaval transposition (a model of portal hypertension, a consequence of liver congestion) prevents the induction of tolerance that otherwise follows the oral administration of antigens.

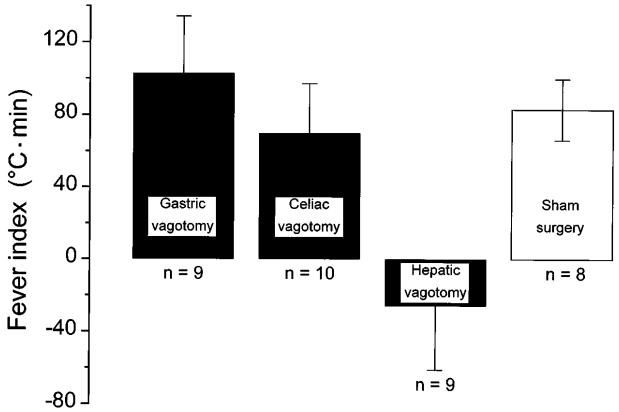

Fig. 3. Febrile response to LPS (1 μg/kg iv) is compared between 4 indicated groups of rats by using fever index (calculated over 0- to 4-h period).

The current hypothesis (for review, see Refs. 7, 34) states that chemical signals initiating a febrigenic afferentation to the brain (these possibly include IL-1 and other pyrogenic cytokines) originate in Kupffer cells of the liver and bind to the appropriate receptors on the vagus, the hepatic vagus, as we know now. Recent studies with gadolinium chloride (in certain experimental paradigms, this drug is thought to selectively inactivate Kupffer cells) confirm the involvement of Kupffer cells in the pathogenesis of both fever (29) and LPS-induced hyperalgesia (36). The participation of the hepatic vagus in pyrogenic signal processing is also supported by some data: *1*) infusion of IL-1 into the portal vein increases the discharge rate of hepatic vagal afferents (21), *2*) receptors to IL-1 are present on hepatic vagus-associated paraganglia (14), and *3*) most important, hepatic vagotomy abolishes LPS-induced hyperalgesia (35). In addition, the fact that hepatic

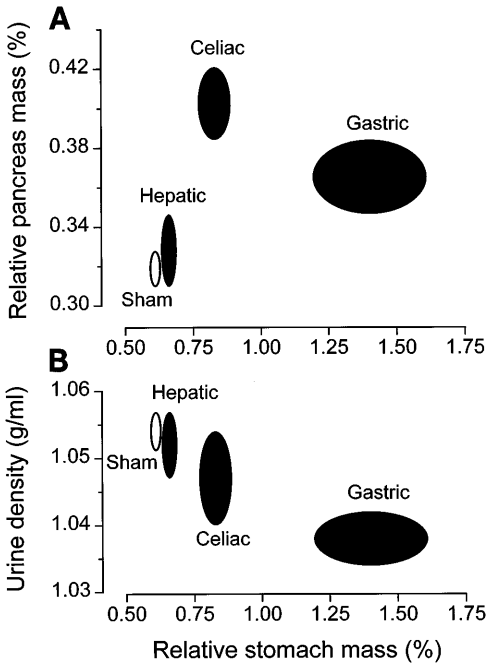

Fig. 4. Separation between 4 groups of vagotomized rats in a 3-dimensional space (stomach mass, pancreas mass, and urine density) is shown, for simplicity, as a set of 2 plane graphs. For each group of animals, data are presented as an oval. Ordinate and abscissa of center of each oval in *A* are mean pancreas mass and stomach mass, respectively. Ordinate and abscissa of center of each oval in *B* are mean urine density and stomach mass, respectively. Vertical and horizontal diameters of ovals are each equal to double SE of corresponding mean.

afferent fibers originate almost exclusively in the portal vein and periportal parenchyma (6), i.e., in the area that harbors the vast majority of Kupffer cells, points to the periportal zone as the site for interactions between potentially pathogenic materials (coming from the gut with the portal circulation), Kupffer cells, and hepatic afferents. Finally, the present data crown the list of evidence (mostly circumstantial) by demonstrating the importance of the integrity of the hepatic vagal branch for fever pathogenesis in a direct experiment.

Our discussion would be incomplete if we did not mention a finding that seems contradictory: hepatic vagotomy had no effect on the thermal responsiveness of rats to intraperitoneal IL-1 in a study by Watkins et al. (33). However, there is no real contradiction. A low dose of LPS injected intravenously (present study) is likely to be taken up by the liver; when the liver is selectively denervated, neural signals from this organ cannot reach the brain and cannot trigger the appropriate responses. On the other hand, when IL-1 is injected intraperitoneally (33), it has access to receptors in a wide spectrum of intra-abdominal organs and tissues, and the transection of any single vagal branch may not have significant functional consequences.

Perspectives

The concept of vagal participation in conveying pyrogenic messages from the periphery to the brain has substantial support, but some of the supporting data remain contradictory. As an example, several investigators have reported that subdiaphragmatic truncal vagotomy does not affect the febrile response to relatively high doses of extraperitoneal (e.g., intravenous or intramuscular) pyrogens (8, 15, 26), whereas others demonstrated nearly complete blockade of the biphasic response to intravenous LPS by either subdiaphragmatic vagotomy (28) or pretreatment of intra-abdominal afferent fibers with capsaicin (32). These discrepancies may be rooted in methodological differences such as the method of vagal deafferentation or the postoperative animal care. It is well understood that each method of deafferentation (truncal vagotomy, selective subdiaphragmatic vagotomy, capsaicin desensitization, and vagal rhizotomy) has unique pathophysiological consequences that may alter experimental results. What is less appreciated is that these side effects of vagal deafferentation may be profoundly modified by animal care, especially postoperative nutrition. The dietary digestibility and metabolizable energy density affect the general health status, metabolic state, and postsurgical survival of vagotomized animals (2). In our recent studies (24, 25), rats that were well-nourished after bilateral subdiaphragmatic truncal vagotomy exhibited apparently normal cold-defense responses. This is in contrast to earlier reports by others, who found that subdiaphragmatic vagotomy caused thermoeffector incompetence, ranging from mild (2, 3) to severe (17). Thus the same vagal deafferentation technique may affect thermoregulatory responsiveness differently, depending on the animal's nutritional and health status. Therefore, we recommend that data from vagal deafferentation studies be evaluated paying close attention not only to side effects of the procedure per se, but also to how these side effects are modified by animal care.

As for the present study, the principal results were obtained using selective transection of the hepatic branch; no emaciation or other substantial side effects were observed. This is not a surprise, because the hepatic branch is the second smallest among the major branches of the abdominal vagus and contains by far the lowest proportion of efferent fibers (22). Selective hepatic vagotomy is probably the least traumatizing method of vagal deafferentation that has so far been demonstrated to successfully alter the febrile responsiveness.

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