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Brain Fos expression induced by the chemotherapy agent cisplatin in the rat is partially dependent on an intact abdominal vagus

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Abstract

Anticancer agents such as cisplatin stimulate nausea, vomiting, and behaviors indicative of malaise. Rats and mice, and probably all rodents, do not possess a vomiting response, and their ingestion of kaolin clay (a pica response) has been used as an index of malaise. Similar to the action of cisplatin on emesis in vomiting species, in the rat cisplatin activates vagal afferent fibers, and cisplatin-induced kaolin intake is largely dependent on an intact abdominal vagus. Cisplatin also stimulates Fos expression in the rat brain in areas known to play a role in emesis in other species, but it is not known whether vagal input is required for this CNS activation. In the present study, rats were given abdominal vagotomy or sham operation to test the role of an intact vagus on cisplatin-induced Fos expression 6 h after injection with saline or cisplatin (6 mg/kg, ip). Cisplatin treatment produced Fos expression in the area postrema and multiple levels of the nucleus of the solitary tract (NTS) of sham-operated rats. Vagotomy reduced cisplatin-induced Fos expression in the caudal and middle levels of the NTS and central amygdala. Furthermore, cisplatin did not significantly alter Fos expression in the spinal cord (T8–T10) before or after vagotomy. These results suggest that a defined portion of cisplatin-induced Fos expression is dependent on vagal input, with a majority of this response determined by either direct action of cisplatin or humoral factors on the CNS.

Keywords

Vagus; emesis; nausea; pica; Fos

1. Introduction

Nausea and vomiting are among the most common side effects of chronic disease and pharmacological treatments. Some cancer chemotherapy drugs, such as cisplatin, are extremely potent in producing nausea and vomiting, as well as anorexia, body weight loss, and diarrhea (e.g., Hainsworth et al., 1992; Kris et al., 1988; Kris et al., 2006; Nelson et al., 2002). Despite the use of first-line antiemetic therapies, such as serotonin (5-HT₃) and tachykinin (NK₁) receptor antagonists (Kris et al., 2006), many patients still experience nausea and emesis with chemotherapy (Aapro, 2002; Rudd et al., 2005). Although there is some knowledge of the neural circuits that produce emesis, little is known about neural pathways responsible for nausea (for review, see Horn, 2008). The lack of detailed insight into these neural systems hinders our ability to control nausea and emesis, not only in cancer

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chemotherapy but also in numerous medical conditions, including late-stage cancer and AIDS.

Cats, dogs, ferrets, pigs, shrews, and pigeons have been used to study the neurobiology of emesis and malaise induced by chemotherapy agents (for reviews, see Andrews et al., 2006; Andrews et al., 2004). Although laboratory rats, and likely all rodents, do not have a vomiting response, their ingestion of kaolin clay has been used as a surrogate marker of emesis or malaise (De Jonghe et al., 2008; Liu et al., 2005; Vera et al., 2006), and is inhibited by antiemetic drugs (Rudd et al., 2002; Saeki et al., 2001; Takeda et al., 1995; Takeda et al., 1993). This response might be a strategy to reduce toxicosis, for example, by potentially binding toxins to clay in the gastrointestinal (GI) tract (Phillips, 1999; Phillips et al., 1995), and kaolin intake can help rats recover from illness (De Jonghe et al., 2009b). Cisplatin-induced kaolin consumption in the rat is largely dependent on an intact GI vagus (De Jonghe et al., 2008), and cisplatin produces increased electrophysiological activity in vagal afferents, which is blocked by antagonism of 5-HT₃ receptors (Horn et al., 2004). Although it might also appear useful to analyze conditioned taste aversion (or avoidance) in the context of understanding neural pathways of nausea and emesis, this behavioral metric has proved to be complex and difficult to interpret, with the involvement of other brain systems, for example, gustatory and learning processes (e.g., Huang et al., 2008; Parker, 1995; Rabin et al., 1992; Rudd et al., 1998), that likely have little to do with the direct stimulation of nausea or emesis.

Characterizing the global activation of brain pathways can be partly accomplished by detecting the amount of Fos protein expressed in neuronal cells after stimulation (e.g., Sagar et al., 1988). Cisplatin treatment in vomiting species, such as the cat and ferret, produces Fos expression in the caudal hindbrain, including the nucleus of the solitary tract (NTS) and area postrema (AP) (Ariumi et al., 2000; Miller et al., 1994b; Van Sickle et al., 2003), which receive vagal afferent projections (Norgren et al., 1988; Ranson et al., 1993) and are known to play a role in emesis (for review, see Andrews et al., 2004). Cisplatin-induced Fos expression in the NTS, but not the AP, of the ferret was greatly reduced by cervical vagotomy (Reynolds et al., 1991), which suggests that Fos expression indicates activation of emetic circuits by cisplatin treatment because emesis induced by this drug is also largely dependent on an intact vagus (Andrews et al., 1990). In rats, cisplatin treatment induces Fos expression in the NTS and AP (Horn et al., 2007); however, abdominal vagotomy has no effect, at least qualitatively, on cisplatin-induced c-Fos mRNA expression in the NTS (Endo et al., 2004). This suggests that cisplatin-induced Fos expression in the hindbrain of the vagotomized rat is not sufficient to mediate pica, because vagotomy can substantially reduce cisplatin-induced kaolin intake (De Jonghe et al., 2008).

The present study was designed to further assess the role of the vagus in cisplatin-induced Fos expression in the rat. This was accomplished by quantitative assessment of Fos expression in abdominal vagotomized and sham-operated rats after injection with cisplatin. Hindbrain and forebrain areas, including the NTS (at multiple levels and subnuclei), AP, central amygdala (CeA), and bed nucleus of the stria terminalis (BNST), were analyzed because these CNS regions have been shown to display robust levels of Fos expression after cisplatin treatment (Horn et al., 2007). Fos expression in the spinal cord was also assessed because there are indications from electrophysiological recordings that spinal pathways in the rat become more sensitive to cisplatin after vagotomy (Hillsley et al., 1999) and because lesion experiments in the ferret and cat indicate a role for spinal input in emesis (Andrews et al., 1990; Miller et al., 1994a).

2. Materials and methods

2.1 Animals

Male Sprague Dawley rats (Charles River, Kingston, NY, USA) were housed individually in mesh-floored stainless-steel hanging cages (25 × 18 × 19 cm) and maintained in a temperature-controlled vivarium (~23°C), with a 12/12-h light/dark cycle (lights on at 0600). Animals were handled daily for 2 weeks prior to the onset of experiments. All rats had free access to water and powdered rat chow (LabDiet 5001, PMI Nutrition, Richmond, ID, USA) prior to surgery (see below for protocol after surgery). The protocol was approved by the Monell Chemical Senses Center Institutional Animal Care and Use Committee.

2.2. Vagotomy surgery

Rats were randomly assigned to one of four conditions: sham-saline (n = 5; 389 ± 11 g body weight, mean ± SEM), sham-cisplatin (n = 5; 392 ± 13 g), vagotomy-saline (n = 5; 378 ± 8 g), and vagotomy-cisplatin (n = 6; 378 ± 11 g). After an overnight fast, animals were anesthetized with sodium pentobarbital (50 mg/kg, ip; Sigma-Aldrich, USA), and vagotomies were performed using a thermal cautery according to previously established procedures (De Jonghe et al., 2008; Horn et al., 1996). Briefly, a midline incision was made in the abdomen, the stomach was gently manipulated to reveal the ventral and dorsal trunks of the subdiaphragmatic vagus, and both of these trunks were completely transected in vagotomized animals. Sham-operated rats experienced laparotomy and gentle lifting of the stomach, but cauterization was not applied to any tissue. The peritoneum and abdominal muscles were sutured, and the abdominal skin incision was closed with wound clips, which were removed 7 days after surgery. Postsurgical analgesic (buprenorphine, 0.5 mg/kg, Sigma-Aldrich, USA) and antibiotic (gentamicin, 1 mg/kg, Sigma-Aldrich, USA) were administered twice daily for 2 and 3 days after surgery, respectively. Rats were allowed 14 to 19 days to recover before testing.

2.3. Recovery from vagotomy surgery

Typically, rats with complete subdiaphragmatic vagotomy have gastric stasis, reduced food intake, and lower body weight (e.g., Kraly et al., 1986), which can make results from this surgical treatment difficult to interpret. However, providing a liquid diet instead of solid food can greatly reduce these negative effects of vagotomy surgery (Garcia-Medina et al., 2007; Kraly et al., 1986). In the present study, all animals were given a liquid diet [50% sweetened condensed milk and 0.12% Enfamil Poly-Vi-Sol multivitamin drops (Mead Johnson Nutrition, USA), diluted in water] directly after surgery. Assessment of body weight at 12 days postsurgery revealed no significant difference between assigned groups [$F(3,17) = 1.8$, $P = 0.2$, one-way analysis of variance (ANOVA); sham-saline, 397 ± 14 g body weight, mean ± SEM; sham-cisplatin, 425 ± 23 g; vagotomy-saline, 376 ± 18 g; vagotomy-cisplatin, 371 ± 18 g].

2.4. Drug injection and tissue collection

Rats received two intraperitoneal injections of Fluoro-Gold (Fluorochrome, Denver, CO, USA) for verification of vagotomy (see section 2.6) 4 days prior to assessment of Fos expression. On the day of Fos testing, animals received either saline (0.15 M NaCl) or cisplatin (*cis*-diamineplatinum dichloride; P4394, Sigma-Aldrich, USA) at 0900 – 1030 h, which was injected in a volume of 1.5 ml/kg body weight. Cisplatin was dissolved in saline (0.15 M NaCl), sonicated until clear, and then vortexed immediately prior to injection. Water bottles and food jars were removed 4 h prior to the time of euthanization to minimize contributions of ingestive behavior on Fos expression. The 6 mg/kg dose of cisplatin was chosen based on previous work, which showed that cisplatin produced a robust Fos response

at 6 h postinjection (Horn et al., 2007) and an increase in kaolin intake (De Jonghe et al., 2008).

At 6 h after injection with saline or cisplatin, rats were deeply anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg). The thoracic cavity was opened, and animals were transcardially perfused with 0.2 M phosphate-buffered saline (PBS; pH 7.4), followed by 2% acrolein/4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains and spinal cords were removed and placed in 10% sucrose/PBS followed by 20% and 30% sucrose/PBS, each overnight. After sucrose cryoprotection, brains were frozen using dry ice and subsequently cut at 30 μ m using a cryostat.

Sections were collected from two locations in the brain based on previous work (Horn et al., 2007): i) the caudal hindbrain containing the NTS and AP, and ii) forebrain sections containing the CeA, BNST, paraventricular nucleus (PVN), and supraoptic nucleus (SON). The spinal cord was sectioned at T8 to T10. This level of the spinal cord receives afferent input from the upper GI tract (Qin et al., 2007; Rinaman et al., 2004; Traub et al., 1996). Sections were collected in culture plate wells containing cryoprotectant (Watson et al., 1986) and either stored at -20°C or immediately processed for immunohistochemistry.

2.5. Fos immunohistochemistry

Brain sections were processed similar to previous studies (Horn et al., 1999; Horn et al., 2007). Briefly, after initially rinsing in PBS to remove cryoprotectant, sections were incubated in sequence with 1% sodium borohydride in PBS (20 min), 0.3% hydrogen peroxide in PBS (30 min), and 5% normal donkey serum (NDS) in PBS containing 0.2% Triton X-100 (PBS-TX), with rinses between each step. Sections were then incubated at room temperature with gentle agitation in 1:40,000 polyclonal anti-Fos (Santa Cruz Biotechnology, Santa Cruz, CA, USA; lot no. E1606) containing 1% NDS in PBS-TX for 20 h. Following rinses in PBS-TX, sections were placed in 1:400 biotinylated rabbit anti-rat (Jackson ImmunoResearch, West Grove, PA, USA) for 2 h at room temperature with gentle agitation. This was followed by rinses in PBS and 1 h incubation in avidin-biotin (4.5 μ l avidin and 4.5 μ l biotin per ml PBS; Elite Kit, Vector Laboratories, Burlingame, CA, USA) with gentle agitation. Sections were then rinsed twice in PBS and twice in 0.05 M Tris buffer (pH 7.4). Sections were placed in 3,3'-diaminobenzidine (DAB) in 0.05 M Tris with nickel sulfate (25 mg/ml) for 4–5 min for the chromogen reaction (Elite Kit, Vector Laboratories). Finally, sections were rinsed twice in Tris buffer and twice in PBS, placed on microscope slides, air dried, coated with DPX mountant (Fluka, Ronkonkoma, NY, USA), and cover slipped.

2.6. Verification of vagotomy

Visual verification of subdiaphragmatic vagotomy is not reliable because of growth of connective tissue at the surgical site during recovery. Vagotomy was verified by intraperitoneal injection of the retrograde neuronal tracer Fluoro-Gold (De Jonghe et al., 2008; Horn et al., 1996; Phillips et al., 1998). Four days before Fos testing, rats were given two 0.5-ml intraperitoneal injections of 0.1% Fluoro-Gold. The presence of Fluoro-Gold in the dorsal motor nucleus (DMN) was determined by immunohistochemical labeling as previously described (De Jonghe et al., 2008) using a primary antibody for Fluoro-Gold (1:40,000; lot no. 05023816) and the same protocol described above for Fos immunohistochemistry. Fluoro-Gold staining was analyzed by ImageJ software (NIH; <http://rsb.info.nih.gov/ij/>) to assess the density of staining (gray level) in the left and right DMN relative to the adjacent NTS (higher percentage values indicate darker staining relative to the NTS). See Fig. 1 for representative images of the DMN and comparisons between groups.

2.7. Fos cell counting

Fos cell staining was determined by the presence of a blue-black reaction product in the cell nuclei. Tissue sections were viewed with a Zeiss microscope (Axiostar Plus) equipped with a digital camera (Scion CFW-1312C). Brain regions and cells expressing Fos were imaged, and the number of cells expressing Fos was manually counted without knowledge of the experimental condition using ImageJ software.

Based on previous examinations of brain sections from rats treated with cisplatin, cell counts were made in areas that consistently showed Fos expression (Horn et al., 2007). Areas of the hypothalamus were included in this analysis as controls because these areas tend to display some Fos expression, but it is not inducible by cisplatin treatment (Horn et al., 2007). The spinal cord was counted because reports indicate that cisplatin might activate spinal pathways (Andrews et al., 1990; Hillsley et al., 1999; Miller et al., 1994a). To standardize the analyses, cells from each area were counted in coronal sections from each animal at approximately the same level relative to bregma (Paxinos et al., 2005). Areas of the CNS (Fig. 2) analyzed for Fos expression included the NTS [caudal, -14.7 bregma (NTSc); middle, -13.9 (NTSm); rostral, -13.3 (NTSr)], AP (-13.9), BNST (-0.1), CeA (-2.5), PVN (-1.9), SON (-1.4), and spinal cord (sections from levels T8 – T10). The NTSm was also assessed for Fos expression in the dorsal medial (dm), commissural (com), central (cen), medial (med), and ventral lateral (vl) subnuclei (Phifer et al., 1998). Although it is relatively easy to approximate the location of the dm, com, and vl using the AP, DMN, and solitary tract as landmarks (Fig. 3), it is more difficult to distinguish the cen and med, so these areas were summed together (Horn et al., 2001). Cell counts from the brain were obtained from one to two (consecutive and averaged) sections for each area. For the spinal cord, counts included the average of ~17% of sections from each animal (~55 sections, every sixth serial section). Because Fos expression was not lateralized in any of the bilateral structures examined, cell counts reflect the totals for both sides in these areas. Nickel-enhanced DAB chromogen staining provided some architectural details for the location of brain regions.

Brains were processed in batches, and each batch also included a positive control. Positive controls for Fos expression consisted of brains from rats treated with cholecystokinin (100 μ mol/kg, ip) 1 h prior to euthanasia (e.g., Olson et al., 1992). These positive controls consistently showed a large number of Fos cells in the hindbrain and forebrain. Furthermore, preincubation with Fos protein (sc-52P, Santa Cruz Biotechnology, lot no. J1805) completely blocked Fos staining.

2.8. Statistical analysis

Percentage gray level of Fluoro-Gold in the DMN and Fos cell counts for each brain area were analyzed using 2 (saline or cisplatin) $\times$ 2 (sham or vagotomized) ANOVA. Planned comparisons were conducted between saline versus cisplatin for sham and vagotomy conditions using least significant difference (LSD) tests. For all analyses, a P-value of 0.05 was used to determine statistical significance. Group values are reported as the mean $\pm$ SEM. Statistical analyses were conducted using Statistica (version 8, StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Verification of vagotomy

Complete subdiaphragmatic vagotomy substantially reduced the labeling of the Fluoro-Gold tracer in the DMN (Fig. 1A). The percentage gray level of the DMN relative to the adjacent NTS was significantly different between sham and vagotomized animals (Fig. 1B). There

was no significant interaction of vagotomy and cisplatin treatment ($P > 0.05$), but labeling was significantly less in vagotomized animals [$F(1,17) = 27.7$, $P < 0.0001$, main effect of vagotomy]. For saline and cisplatin treated animals, vagotomy produced less Fluoro-gold labeling in the DMN (LSD tests, Fig. 1). For percentage gray level in the DMN relative to the NTS, all vagotomized animals had a negative value, and all sham animals had a positive value, i.e., there was no overlap in the distribution of data between sham and vagotomized animals.

3.2. Fos expression in the hindbrain and spinal cord

Cisplatin produced Fos expression in the NTSc, NTSm, NTSr, and AP [all $F(1,17) \geq 8.5$, all $P \leq 0.01$, cisplatin main effect for each brain region; Fig. 4]. There were no significant interaction effects for vagotomy by injection (ANOVAs, interaction effects, all $P > 0.05$). There were also no significant effects of injection or vagotomy on Fos expression in the DMN or the spinal cord (ANOVAs, main and interaction effects, all $P > 0.05$). Planned comparisons revealed that both sham-operated and vagotomized animals showed cisplatin-induced Fos expression in the NTSr and AP, but only sham-operated animals showed cisplatin-induced Fos expression in the NTSc and NTSm (LSD tests; Fig. 4). There were no significant effects of Fos expression in the DMN or spinal cord using planned comparisons (LSD tests; Fig. 4). Fig. 4 reports the average number of cells per spinal cord section. Spinal cord data from one animal in the sham-saline group was not used because only a few tissue sections were obtained after immunohistochemical processing. Spinal cord data were also analyzed by using the total Fos cells for all sections, the top 5 or top 20 cell counts from each animal, counts from dorsal horn areas 1 to 5, or counts from area 7 and the intermediate zone (Paxinos et al., 2005), however, these alternate ways did not produce statistically significant differences between groups (ANOVA and planned comparisons, all $P > 0.05$; data not shown).

3.3. Fos expression in the subnuclei of the NTS

Cisplatin produced Fos expression in the dm and cen-med subnuclei [all $F(1,17) \geq 5.8$, all $P \leq 0.05$, cisplatin main effect for each brain region; Fig. 5]. There were no significant interaction effects for vagotomy by injection (ANOVAs, interaction effects, all $P > 0.05$). Although there was no statistically significant main effect of cisplatin treatment on Fos expression in the com subnucleus ($P = 0.052$), a planned comparison showed that cisplatin treatment produced an increase in Fos expression in sham-operated but not vagotomized animals (LSD tests; Fig. 5). Furthermore, planned comparisons showed a statistically significant increase in cisplatin-induced Fos expression in the dm subnucleus in sham-operated but not vagotomized animals (LSD tests; Fig. 5).

3.4. Fos expression in the forebrain

Cisplatin produced Fos expression in the BNST and CeA [all $F(1,17) \geq 45.1$, all $P \leq 0.00001$, cisplatin main effect for each brain region; Fig. 6]. Furthermore, there was an interaction effect of injection and surgery for Fos expression in the CeA [$F(1,17) = 6.0$, $P < 0.05$, injection by surgery interaction effect; Fig. 6]. Planned comparisons revealed that cisplatin treatment produced an increase in Fos expression in sham-operated and vagotomized animals for both the BNST and CeA (LSD tests; Fig. 6). There were no statistically significant effects of cisplatin treatment or vagotomy on Fos expression in the SON or PVN [data not plotted; ANOVA, all $P > 0.05$; SON, sham-saline (2.1 ± 1.3), sham-cisplatin (0.2 ± 0.1), Vx-saline (1.5 ± 0.9), Vx-cisplatin (24.2 ± 22.9); PVN, sham-saline (120.4 ± 35.5), sham-cisplatin (150.6 ± 6.2), Vx-saline (97.9 ± 20.9), Vx-cisplatin (152.1 ± 16.9)].

4. Discussion

Intraperitoneal injection of cisplatin produced Fos expression in the AP and multiple levels of the NTS in sham-operated rats. Abdominal vagotomy reduced the effect of cisplatin-induced Fos expression in NTSc, NTSm, and the CeA. Furthermore, cisplatin did not significantly stimulate Fos expression in the spinal cord (T8 – T10) before or after vagotomy. These results suggest that only a specific component of cisplatin-induced brain Fos expression is dependent on abdominal vagal input, with a majority of this response determined by either direct action of cisplatin or humoral factors released by cisplatin on the CNS.

The dose of cisplatin and time point of Fos expression used in the present report were determined by prior studies (De Jonghe et al., 2008; Horn et al., 2007; Malik et al., 2007; Malik et al., 2006). A 6 mg/kg dose of cisplatin produces increased brain Fos expression, increased consumption of kaolin, and reduced food intake (De Jonghe et al., 2008; Malik et al., 2007; Malik et al., 2006), and 6 h after injection with this dose, intense expression of Fos appears in the CNS (Horn et al., 2007). This dose also produces hindbrain and forebrain Fos expression at 24 h, although less than at 6 h, and in the BNST at 48 h after injection (Horn et al., 2007); this present study did not assess the role of the vagus in the delayed phase of cisplatin-induced Fos expression. Chemotherapy-induced nausea and emesis are typically divided into acute (< 24 h) and delayed (> 24 h) phases. These phases appear to have distinct mechanisms, because 5-HT₃ receptor antagonism is most effective for inhibiting acute-phase nausea and emesis, while NK₁ receptor antagonists work best to control the delayed phase (Kris et al., 2006). Acute-phase vomiting and pica produced by cisplatin treatment are substantially reduced by vagotomy (De Jonghe et al., 2008; Sam et al., 2003); therefore, if cisplatin-induced Fos expression is related to the behavioral effects of this drug, vagotomy should reduce Fos labeling at 6 h after injection with cisplatin.

In a prior study, abdominal vagotomy was reported to have no effect on cisplatin-induced c-Fos mRNA expression in the NTS or AP (Endo et al., 2004); however, that study differed from the present experiment in several ways. The earlier report did not present quantitative information, but included representative images of labeling for c-Fos mRNA (Endo et al., 2004), so it is unclear whether there were less prominent statistical differences between hindbrain Fos expression in sham-operated and vagotomized animals. Furthermore, a 2 h postinjection time point was used to assess c-Fos mRNA in the prior study, which likely reflects Fos protein expression that might occur ~45 min after the 2 h time point (Sharp et al., 1991). Based on the long time course of cisplatin-induced brain Fos expression—at least 48 h (Horn et al., 2007)—this time difference between the prior study and the 6 h time point used in the present study is unlikely to be a significant factor. Pretreatment with the 5-HT₃ receptor antagonist granisetron was also reported to substantially reduce cisplatin-induced Fos expression in the NTS (Endo et al., 2004). Antagonists of 5-HT₃ receptors might target 5-HT₃ receptors on vagal afferent fibers (Horn et al., 2004) or hindbrain areas, such as NTS and AP (Jeggo et al., 2005; Kilpatrick et al., 1989), to diminish Fos expression. Although vagal afferent fibers containing 5-HT₃ receptors are a major contributor of cisplatin-induced activation of emesis (e.g., Andrews et al., 1990), it is clear from the present and prior report (Endo et al., 2004) that cisplatin-induced brain Fos expression in the NTS and AP is largely independent of an intact abdominal vagus.

The present study suggests a restricted effect of vagotomy on cisplatin-induced Fos expression in the hindbrain. The effect of vagotomy to reduce cisplatin-induced Fos expression appeared to be isolated to more medial and caudal regions of the NTS. An analysis of NTSm subnuclei revealed that lesion of the vagus produced a reduction in cisplatin-induced Fos labeling in the dm and com subnuclei, which border the AP. Notably,

the more medial segments of the NTS receive the densest projection from vagal afferents (Rinaman et al., 2004).

Although there are distinct muscular characteristics of rodents that make vomiting difficult to accomplish, for example, a thin and long esophagus (Andrews, 1995), there might also be differences in neural circuitry between vomiting and nonvomiting species. We recently reported intense Fos expression in the DMN 6 h after injection with cisplatin in the house musk shrew, a vomiting species (De Jonghe et al., 2009a). DMN neurons have output to multiple levels of the GI tract (Won et al., 1998) and might initiate the giant retrograde contraction to move intestinal contents toward the gastric compartment in preparation for expulsion. Furthermore, the DMN is implicated in the control of the crural diaphragm (Niedringhaus et al., 2008a; Niedringhaus et al., 2008b), which is an important muscular component of emesis (Grelot et al., 1994). The current study and a prior one (Horn et al., 2007) show that Fos expression in the DMN of the rat was unaffected by cisplatin treatment.

It was previously demonstrated using electrophysiological methods that non-vagal intestinal afferent fibers, possibly spinal, become more sensitive to cisplatin after abdominal vagotomy (Hillsley et al., 1999). Although not statistically significant, in the present study there was a trend for cisplatin to produce Fos expression in the spinal cord after vagotomy (Vx-saline vs. Vx-cisplatin planned comparison, $P = 0.08$), which suggests a degree of plasticity in the cisplatin-response in spinal pathways. The amount of Fos expression in vagotomized animals treated with cisplatin, if it is reliable, suggests that this effect is relatively small in comparison to effects of cisplatin on Fos expression in the hindbrain.

Similar to our prior study (Horn et al., 2007), cisplatin treatment produced a large Fos response in the CeA and BNST, with no significant difference in the levels of Fos expression in the hypothalamic areas of the PVN and SON. A lack of a Fos response in the PVN and SON after cisplatin treatment in this and prior work (Horn et al., 2007) is in sharp contrast to the enhanced Fos expression observed with different stressors, such as osmotic challenge, immobilization, and pain (for review, see Pacak et al., 2001). Cisplatin appears to produce activation of the forebrain that is markedly different from agents that, acting on the gut-brain axis, produce stress and pain, and therefore might provide insight into the neural systems responsible for nausea and malaise. Based on the present results, the CeA, BNST, NTS, and AP appear sensitive to cisplatin treatment. A reduction in cisplatin-induced Fos expression in the CeA after vagotomy suggests that this region is part of a gut-brain pathway sensitive to cisplatin. The BNST receives extensive projections from the CeA, shares similar neurotransmitter profiles as the CeA, and has been considered to be a key component of an extended amygdala (de Olmos et al., 1999; Heimer et al., 2006; Swanson, 2003). The CeA and BNST are highly differentiated nuclei that receive inputs from cortical areas involved in conscious perception, for example, prefrontal, chemosensory (i.e., olfactory, gustatory), and viscerosensory cortices. Therefore, the CeA and BNST are strategically located to act as important relays for autonomic, emotional, and neuroendocrine signals involved in feeding, anorexia, and probably nausea. Cisplatin treatment also produced Fos expression in the CeA in the house musk shrew, a vomiting species (De Jonghe et al., 2009a).

A significant part of cisplatin-induced brain Fos expression in the present study was independent of an intact abdominal vagus. Although cisplatin does not appear to cross the blood-brain barrier (Litterst et al., 1979), it might potentially act on the AP, which is outside this boundary. The AP receives vagal afferent input, although less than the NTS (Norgren et al., 1988; Ranson et al., 1993), and it is difficult to interpret lesions or chemical infusions into this area because these manipulations could also affect vagal afferent signaling. With this caveat in mind, ablation of the AP or discrete injections of a 5-HT₃ receptor antagonist

can inhibit cisplatin-induced emesis (Higgins et al., 1989; McCarthy et al., 1984). Cisplatin could also cause the release of humoral factors, possibly from the GI tract, which could act on the brain. These humoral factors might include 5-HT and substance P, because evidence shows that these agents, or their breakdown products, are elevated in the blood after cisplatin treatment (Higa et al., 2006).

In summary, the present report shows that cisplatin-induced brain Fos expression in the rat is partly dependent on an intact abdominal vagus. Abdominal vagotomy resulted in reductions in Fos labeling in caudal and medial divisions of the NTS and in the CeA 6 h after treatment with 6 mg/kg cisplatin. It remains to be determined which sub-branches of the abdominal vagus are responsible for this effect and what are the phenotypes of brain cells activated by cisplatin and dependent on an intact vagus. The present study is important because it helps to define neural systems involved in malaise, which can significantly affect feeding behavior in patients with chronic disease, such as cancer and AIDS, who receive potent drug treatments. Also of particular relevance is the distinction between the neural systems for malaise in common laboratory rodents, which lack a vomiting response, and emetic species, such as the ferret, cat, dog, and musk shrew. It can sometimes be difficult to determine when rodents are experiencing malaise, and the extent to which these systems are defined helps to assess malaise in nonvomiting species and to distinguish activation of these neural pathways from other brain systems, such as those involved in the control of feeding behavior.

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Fig. 1. Verification of subdiaphragmatic vagotomy using retrograde tracing. Animals received an intraperitoneal injection of the retrograde neuronal tracer Fluoro-Gold. Hindbrain sections containing the dorsal motor nucleus (DMN) were immunohistochemically labeled for Fluoro-Gold. **A:** Hindbrain sections from a sham-operated (left) and a vagotomized (Vx, right) rat labeled for Fluoro-Gold. The left section shows the regions that were used to determine the gray level of the DMN relative to the nucleus of the solitary tract (NTS). **B:** Percentage gray level in the DMN relative to the NTS in the four study conditions. * $P < 0.05$, LSD tests, sham-saline versus vagotomy-saline, or sham-cisplatin versus vagotomy-cisplatin.

Fig. 2. Location of Fos cell counting in a sham-operated rat injected with cisplatin (6 mg/kg, ip). Areas enclosed by dashed lines indicate the location of counting in the bed nucleus of the stria terminalis (BNST) and central nucleus of the amygdala (CeA); nucleus of the solitary tract (NTS; r, rostral; m, middle; c, caudal), area postrema (AP), and dorsal motor nucleus (DMN); and thoracic levels (T8 – T10). Small darkly stained Fos-positive cells are prominent in the NTS and AP. Outlined areas in the forebrain [lateral ventricle (lv), internal capsule (ic), anterior commissure (ac), and optic tract (ot)] are shown for reference.

Fig. 3. Location of Fos cell counting in subnuclei of the middle nucleus of the solitary tract (NTSm; see Fig. 2) in a sham-operated rat injected with cisplatin (6 mg/kg, ip). The NTS subnuclei include the dorsomedial (dm), commissural (com), central (cen), medial (med), and ventral lateral (vl). The area postrema (AP), solitary tract (ts), dorsal motor nucleus (DMN), and central canal (c) are shown for reference. Darkly stained Fos-positive cells are prominent in the NTS and AP.

Fig. 4. Fos cell counts (mean $\pm$ SEM) from the hindbrain and spinal cord 6 h after injection with saline or cisplatin (6 mg/kg; ip), after vagotomy (Vx) or sham operation: nucleus of the solitary tract (NTS; c, caudal; m, middle; r, rostral), area postrema (AP), dorsal motor nucleus (DMN), and spinal cord levels T8 – T10 (Spinal). *P < 0.05, LSD test, cisplatin compared with saline injection.

Fig. 5.

Fos cell counts (mean $\pm$ SEM) from subnuclei of the middle level of the nucleus of the solitary tract (NTSm; see Figs. 2 and 3) 6 h after injection with saline or cisplatin (6 mg/kg; ip), after vagotomy (Vx) or sham operation. The NTS subnuclei include the commissural (com), dorsomedial (dm), central and medial (cen-med), and ventral lateral (vl). * $P < 0.05$, LSD test, cisplatin compared with saline injection.

Fig. 6. Fos cell counts (mean $\pm$ SEM) from the bed nucleus of the stria terminalis (BNST) and central nucleus of the amygdala (CeA) 6 h after injection with saline or cisplatin (6 mg/kg; ip), after vagotomy (Vx) or sham operation. * $P < 0.05$, LSD test, cisplatin compared with saline injection.