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Disruption of re-intake after partial withdrawal of gastric food contents in rats lesioned in the gelatinous part of the nucleus of the solitary tract

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ABSTRACT

Sensory information from the upper gastrointestinal tract is critical in food intake regulation. Signals from different levels of the digestive system are processed to the brain, among other systems, *via* the vagus nerve, which mainly projects towards the nucleus of the solitary tract (NST). The objective of this study was to analyze the participation of the gelatinous part (SolG) of the NST in short-term food intake. One-third of the stomach food content was withdrawn at 5 min after the end of a meal, and food was then available *ad libitum* for different time periods. SolG-lesioned and control animals ingested a similar amount of the initial liquid meal, but the former consumed significantly smaller amounts and failed to compensate for the food deficit, whereas the controls re-ingested virtually the same amount as extracted. These data suggest that the SolG, as in the case of related anatomical structures such as the vagus nerve or external lateral parabrachial subnucleus, may be relevant in particular circumstances that require the rapid processing of vagal-related food intake adjustment associated to the upper gastrointestinal tract.

1. Introduction

Study of the mechanisms involved in the control of food intake (meal size) has been of special interest over the years due to their potential importance in the treatment of obesity, a major public health problem in developed countries (Page, Symonds, Peiris, Blackshaw, & Young, 2012; Folgueira, Seoane, & Casanueva, 2014; D'Agostino et al., 2016).

Information generated by food in the upper gastrointestinal tract appears to be crucial in meal size control (Powley & Phillips, 2004; Roman, Derkach, & Palmiter, 2016; Schwartz, 2006). These mechanical (volumetric) and chemical data are largely transmitted to the brain *via* the vagus nerve (Phillips & Powley, 1998; Zafra, Molina, & Puerto, 2003), whose afferents project almost exclusively, and with a certain viscerotopic organization, to the nucleus of the solitary tract (NST) (Altschuler, Bao, Bieger, Hopkins, & Miselis, 1989; Barraco, el-Ridi, Ergene, Parizon, & Bradley, 1992; Gieroba & Blessing, 1994), a gateway of visceral signals to the brain (D'Agostino et al., 2016; Roman et al., 2016). It has been demonstrated that the densest concentration of gastric vagal afferents is in the lateral portion of the dorsomedial part of the intermediate-caudal region of the NST, known as the gelatinous subnucleus (SolG) (Altschuler et al., 1989; Barraco et al., 1992; Herbert, Moga, & Saper, 1990; Rinaman, Card, Schwaber, & Miselis, 1989; Shapiro & Miselis, 1985). However, in contrast to other parts of the dorsomedial subnucleus, the SolG receives few intestinal projections, which are preferentially distributed in more caudal regions (Barraco et al., 1992; Zhang et al., 1995, 1992; Zittel, De Giorgio, Sternini, & Raybould, 1994).

C-fos activity has been observed in specific subnuclei of the intermediate-caudal region of the NST (NSTic) after the normal intake of a meal (Emond, Schwartz, & Moran, 2001; Fraser & Davison, 1993; Gaykema et al., 2009; Olson et al., 1993; Rinaman, Baker, Hoffman, Stricker, & Verbalis, 1998), after the direct administration of nutrients in different digestive segments (Emond et al., 2001; Phifer & Berthoud, 1998; Wang, Cardin, Martínez, Taché, & Lloyd, 1999; Yamamoto & Sawa, 2000a,b; Zittel et al., 1994; Mönnikes et al., 1997) and in response to certain intake-related stimuli, including gastric distension (Gonzalez, Sharp, & Deutsch, 1986; Olson et al., 1993; Fraser et al., 1995; Zhang et al.,

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1995; Willing & Berthoud, 1997; Emond et al., 2001; Mazda, Yamamoto, Fujimura, & Fujimiya, 2004; van de Wall, Duffy, & Ritter, 2005), intestinal distension (Zhang, Fogel, & Renehan, 1992, 1995, 1998), and peripheral peptide secretion/administration (Fraser & Davison, 1992; Olson et al., 1993; Yang et al., 2004; Li & Rowland, 1995; van de Wall et al., 2005). This effect has even been recorded in response to aversive visceral stimuli (Mediavilla, Bernal, & Puerto, 2007, Yamamoto & Sawa, 2000 a,b). In many of these cases, NST activation can be abolished by chemical or surgical lesions of vagal afferents (Fraser & Davison, 1992; Li & Rowland, 1995; Mönnikes et al., 1997; Yamamoto & Sawa, 2000a; Mazda et al., 2004; Yang et al., 2004; van de Wall et al., 2005).

Hence, the NST is known to be involved in processes related to nutrient intake, and lesions of certain NST subnuclei have been found to trigger the overconsumption of preferred foods (South & Ritter, 1983), to reduce nutrient intake (Menani, Colombari, Talman, & Johnson, 1996), to block the effects on intake of some food-related drugs (Treece, Ritter, & Burns, 2000), and to interrupt taste aversion learning (Mediavilla, Bernal, Mahía, & Puerto, 2011).

With this background, the objective of the present study was to examine the relevance in short-term food intake regulation of the SolG, one of the subnuclei of the intermediate-caudal region of the NST, as noted above, by investigating re-intake behavior after the removal of part of the gastric content immediately after ending a test meal (satiation). In these conditions, neurologically intact animals habitually consume food until they recover approximately the same amount as extracted (Snowdon, 1970; Davis & Campbell, 1973; Deutsch, Young, & Kalogeris, 1978; Zafra, Molina, & Puerto, 2016a,b). Our hypothesis was that animals with SolG lesions would consume a significantly lower amount in comparison to non-lesioned controls and, as shown in related studies (Zafra et al., 2016a,b), would be unable to compensate for the deficit created, because they lack the vagal information required for the correct regulation of this behavior.

2. Materials and methods

2.1. Subjects

Twenty-four adult male Wistar rats (286–334 g at time of surgery), randomly assigned to two groups (SolG-lesioned group: N = 12; control sham-lesioned group: N = 12), were used in this experiment. The animals were individually housed in 30 × 15 × 30 cm methacrylate cages with free access to water and pellet stock diet (Panlab, S.L. Barcelona). The laboratory was maintained under a 12/12 h light-dark cycle (lights on 08:00 h) and at a temperature of 22 ± 1 °C. All experimental procedures took place during light periods and were conducted in accordance with the Animal Care and Use Guidelines established by European Community Council Directive (86/609/CEE) and Spanish legislation (Royal Law 1201/2005). All efforts were made to minimize animal suffering and the number of animals used.

2.2. Surgical procedure

2.2.1. SolG lesions

Surgery was carried out under general anesthesia with sodium pentothal (50 mg/kg, ip; B Braun Medical S.A. Barcelona, Spain) with the animals placed in a stereotaxic unit (Stoelting Co. Stereotaxic 51.600). An incision approximately 1.5 cm in length was made in the upper area of the cranium, connective tissue adhered to the cranium was removed, and two small trephine holes were drilled at the anteroposterior and lateral coordinates corresponding to the SolG. The *dura mater* was then sectioned, and a 00 monopolar stainless steel electrode (approximately 200 μm in diameter and insulated throughout its length except at the tip) was introduced until it reached the dorsoventral coordinate. The electric circuit was then completed by a mass electrode placed in the periphery of the animal, and a cathodic electric current (0.3 mA) was bilaterally applied for 10 s with a DCML-5 lesion-maker (Grass Instruments Corp., Quincy, Mass, USA). The anatomical coordinates (interaural references) for the SolG, obtained from the Paxinos and Watson stereotaxic atlas (1996), were: anterior/posterior (AP) = −4.3 mm; lateral (L) = ± 0.9 mm; and dorsoventral (V) = +2.3 mm.

All of the above steps were followed for the sham lesion control group except that a dorsoventral coordinate of +2.9 mm was used and no current was applied.

2.2.2. Intragastric catheter

After the brain surgery, an intragastric catheter was implanted following a procedure reported elsewhere (Deutsch & Koopmans, 1973; Zafra et al., 2016a,b). A laparatomy of approximately 3 cm was performed, and the stomach was carefully pulled out from the abdominal cavity. An incision of approximately 2 cm was made in the cardia region at the greater curvature, through which a silastic tube was inserted (ID = 1.0 mm; OD = 2.0 mm). Around the end of the silastic tube, a small silicone protuberance was performed to prevent outward displacement of the catheter once the incision was closed around it. Closure was accomplished with a suture around the stomach tissue surrounding the catheter at its insertion site. In addition, the catheter was anchored to the stomach by making a suture point on the surface of the gastric tissue with the remaining suture thread. The exteriorized organs were kept continuously irrigated with isotonic physiological serum (Apiroserum. Lab. YBIS, Madrid, Spain) throughout this procedure. Next, the stomach was returned to the gastric cavity in its original position, and the catheter was routed through the abdominal muscle wall and tunneled subcutaneously to the dorsal surface behind the neck. Stitching was performed as needed to close the wounds, the catheter was capped to avoid gastric content leaking, and silicone was applied around the tip of the catheter to prevent its displacement within the subcutaneous tunnel. As prophylactic measures against infection, povidone iodine (Betadine, Asta Médica, Madrid, Spain) was topically applied to the wounds, and 0.1 cc penicillin (10,000 U; Penilevel Retard. Lab., Level, S.A. Barcelona) was intramuscularly injected.

2.3. Behavioral procedure

The behavioral procedure began seven days before the surgery (see Table 1). During the first five days of this period (Table 1: days −7 to −3), rats were adapted to consume a liquid diet (chocolate-flavored milk, Puleva Food, S.L., Granada; 100 ml contains 12.2 g carbohydrates, 2.2 g fat, and 3 g protein; total energy = 81 Kcal). On the morning of the first day of this 5-day period (10:00), animals were deprived of food and water and then, at the end of the afternoon (18:00), were presented with the liquid diet for the first time (during 1 h). On days −6 and −5, the diet was offered at 10:00 and 12:30 for 30 min (except for the first session on day −6, when it was offered for 60 min). On days −4 and −3, this diet was offered for only 30 min (at 10:00). On days −6 to −4, water was offered for 10 min at around 30 min after finishing the food ingestion session, followed by a pellet stock diet (7.5 g on days −6 and −5; 10 g on day −4) (Table 1). On days −3 to −1, solid food (pellet stock diet) and water were available *ad libitum* (on day −3 after consumption of the liquid diet).

On day 0, the rats underwent surgery (SolG-lesion/Sham-lesion and intragastric catheter). During this day and the next three days (recovery period: days 1–3 in Table 1), a diet of solid food (pellet stock diet) and tap water was available *ad libitum*, and the amount of