Gastric Emptying and Gastrointestinal Transit of Liquid Throughout the First Month After Thoracic Spinal Cord Transection in Awake Rats

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Spinal cord transection (SCT) inhibits gastrointestinal motility in awake rats. We studied the gastric emptying (GE) and gastrointestinal transit of liquid throughout the first month after thoracic SCT. Male Wistar rats (N = 66) were submitted to laminectomy followed or not by complete SCT between T4 and T5 vertebrae. Phenol red recovery in the stomach, proximal, mid- and distal small intestine was determined 1, 7, 10, 15, and 30 days thereafter. Gastric recovery increased by 51.2 and 38.9% and mid-intestinal recovery decreased by 45.5 and 66.6% at one and seven days after SCT (P < 0.05). Proximal small intestine recovery increased by 45.9% 10 days after SCT but no inhibition of gastrointestinal motility was observed thereafter. Stool output significantly decreased in the first seven days after SCT (P < 0.05). In summary, gastrointestinal motility in awake rats is inhibited throughout the first 10 days after thoracic SCT but not thereafter.

KEY WORDS: spinal cord injury; gastric emptying; gastrointestinal motility; rats.

Spinal cord injury (SCI) markedly alters autonomic nervous system function. It is immediately followed by spinal shock, which is characterized by autonomic and somatic hypo- or arreflexia (1). Subsequently, a new phase, characterized by exaggerated reflex responses replaces the spinal shock phase.

The impact of the SCI on gastrointestinal motility is still not totally understood and could only be more clearly appreciated once major advances in all medical fields prolonged the survival of SCI patients. In humans, delayed colonic transit (2), anorectal dysmotility (3), and a major impact of gastrointestinal disorders on the quality of life have been demonstrated (4). However, the effect of SCI on gastric emptying (GE) is still controversial. Delayed GE in the chronic phase has been reported (5–10) but subsequently questioned (11, 12), which may be explained by differences in age, duration, completeness, and mechanism of injury among the different series.

In animals, colonic motor activity seems to be decreased in awake rats (13), but the intestinal myoelectric activity is not affected by spinal cord transection.
(SCT) in anesthetized rats (14). We have also observed that cervical or thoracic SCT inhibits GE and gastrointestinal transit of liquid in awake rats during the first week after SCT (15, 16). In this study, we studied GE and gastrointestinal transit of liquid throughout the first month after thoracic SCT in awake rats. Part of this work has been reported in abstract form elsewhere (17).

MATERIALS AND METHODS

Animals, Protocol of SCT, and Experimental Design. Experiments were performed on 66 male Wistar rats, weighing 160–210 g. All surgical procedures and animal treatments were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals” [DHEW Publication No (NIH) 85-23, Bethesda, Maryland, USA]. The animals were deprived of food for 16 hr but water was allowed ad libitum until 2 hr prior to the surgery. They were then anesthetized with ether and, after laminectomy, the fourth (T4) and fifth thoracic (T5) vertebrae were carefully exposed via a midline dorsal incision and the SCT performed, using a fine cut scissor. Sham-operated animals also underwent laminectomy but without subsequent cord transection (13). Bleeding was minimal and usually stopped in 10–15 sec. The completeness of the SCT was verified by careful inspection of the lesion with the aid of a 10× lens coupled to an optic light. A complete transection was confirmed in all cases, and the other clinical parameters (paraplegia, lack of nociception and somatic reflexes below the lesion as well as urinary retention) indicating completeness of the SCT were observed after recovery from anesthesia. Immediately after SCT, the rats were allowed to recover on a warm pad and were closely monitored for signs of respiratory or circulatory distress. Usually, the rats were awake and mobile (using forelimbs) 20 min after surgery. We considered healthy spinal rats those animals that exhibited grooming and exploratory behavior when removed from their cage, according to Osborn et al (18). All animals received daily subcutaneous antibiotic infusion (procain penicillin, 30,000 IU/kg). In the first few days after surgery, normal saline was administered together with the antibiotics to provide initial hydration. Animals were placed in separate cages and maintained with water ad libitum. Bladder emptying was initially accomplished by manual compression three to four times a day (19). However, 10 days after SCT, almost all animals required manual compression only twice a day.

Measurement of GE and Gastrointestinal Transit. The animals were deprived of food again one day prior to the GE and gastrointestinal transit measurements, but allowed water ad libitum until 2 hr before these measurements, which were performed 1, 7, 10, 15, and 30 days after surgery. For GE and gastrointestinal transit measurements, we used a modification of the technique described by Reynell and Spray (20), which we have previously utilized (21).

First, 1.5 ml of the test meal containing a nonabsorbable marker (0.5 mg/ml phenol red solution in 5% glucose) was given orally by gavage feeding. The animals were killed by cervical dislocation 10 min later. The stomach and small intestine were exposed by laparotomy; quickly clamped at the pylorus, cardia, and terminal ileum; and then removed. The stomach and small intestine from the gastroduodenal junction to the cecum were carefully stretched along a meter-stick on a plain table top and divided into the following four segments: 1, stomach; 2, proximal 40% of small intestine; 3, mid 30% of small intestine; and 4, last 30% of small intestine.

Each segment was placed in a measuring cylinder and the volume measured by adding 100 ml of 0.1 NaOH. They were cut in small pieces and homogenized for 30sec. The suspension was allowed to settle for 20 min at room temperature and 10 ml of the supernatant was centrifuged for 10 min (2800 rpm). Proteins in 5 ml of homogenate were precipitated with 0.5 ml of trichloroacetic acid (20% w/v), centrifuged for 20 min (2800 rpm), and 3 ml from the supernatant was added to 4 ml of 0.5 N NaOH. The absorbance of the sample was read at a wave length of 560 nm and expressed as optical density (OD). A standard dilution curve was obtained in each experiment relating the concentration of phenol red to the optical density of the solution in 0.1 NaOH solution. The linear coefficient of the standard dilution curve (α) was established and used to determine the concentration (C) of the solution read at 560 nm (C = OD) and after the amount of phenol red (m) recovered from each segment (m = C × volume).

The percent recovery of phenol red in each segment (x) was expressed according to the following formula: recovery in segment x = (amount of phenol red recovered in the segment/total amount of phenol red recovered from all four segments).

Each experimental group consisted of five to six animals. We determined the recovery of phenol red at day 1 (N = 5 and 6, respectively for sham-operated and SCT animals), day 7 (N = 5 and 6, respectively for sham-operated and SCT animals), day 10 (N = 5 and 6, respectively for sham-operated and SCT animals), day 15 (N = 5 and 6, respectively for sham-operated and SCT animals), or day 30 (N = 5 and 6, respectively for sham-operated and SCT animals) after thoracic SCT or sham operation. We also performed daily measurements of the weight and stool output of each animal. Sham-operated animals were deprived of food for 12 h every four days in order to avoid major differences in weight between the sham-operated and SCT group.

Cardiovascular Parameters. Mean arterial pressure (MAP) and heart rate (HR) were monitored 1, 7, 10, 15, and 30 days after thoracic SCT. For this purpose, in a separate group of animals, the right carotid artery was cannulated one day prior to MAP and HR measurements. The cannula was then connected to a Narco pressure transducer (P1000B), which was connected to a Mark IV Physiograph (Narco Byo-Systems, Houston, Texas, USA).

Statistical Analysis. The results are expressed as mean ± SEM. One-way analysis of variance (ANOVA) and the Bonferroni test were used to compare the differences in phenol red recovery among the different segments between the various groups. One-way ANOVA for repeated measures followed by the Dunnett’s test was used to compare differences in MAP and HR mean values before and after thoracic SCT. A nonparametric test (Mann-Whitney) was used to compare the difference in stool output among the