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In vitro smooth muscle contractility before and after relief of experimental obstruction in the rat: Application to the surgical management of ileal dilatation

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ABSTRACT

Purpose: Bowel dilatation occurs proximal to an obstruction and predisposes to intestinal dysmotility. The present study sought to determine whether or not changes in smooth muscle contractility and the thickness of the proximal, dilated bowel wall can be reversed following relief of the obstruction.
Materials and methods: Three groups of seven male Wistar rats were studied. In 8-week-old animals in a control group and a sham-operated group, a small segment of bowel (designated as R1 for controls and R2 for shams) was resected 5.0 cm from the cecum. In the third (operated) group, a narrow, isoperistaltic intestinal loop was created proximal to an end-to-end anastomosis of the ileum in 4-week-old animals. When these animals were 6 weeks old, the loop was re-anastomosed to the distal small bowel (after resection of the loop's distal portion, referred to as R3). Two weeks later, a small segment of bowel was resected proximal to the anastomosis (R4). We evaluated the thickness of the smooth muscle layers and the in vitro contractile responses of circular smooth muscle ileal strips (R1–R4) to electrical stimulation and pharmacological stimulation (with KCl, acetylcholine (ACh), substance P, N^G-nitro-L-arginine methyl ester (L-NAME) and histamine).
Results: The amplitudes of contraction in response to electrical and Ach-mediated stimulation were higher for R3 than for R4 ($P < 0.001$), R1 and R2 (both $P < 0.05$). Compared with R1 and R2, the smooth muscle layer was three times as thick in R3 ($P < 0.001$) and 2.5 times as thick in R4 ($P < 0.01$).
Conclusion: Our study provides evidence of the possible recovery of intestinal motility (in response to neurotransmitters involved in gut function) after the relief of an obstruction. If ileal motility can conceivably return to normal values, conservative surgical procedures in pediatric patients should be preferred (in order to leave a sufficient length of bowel and avoid short bowel syndrome).

Ileal dilatation is a common feature of congenital or acquired obstructions encountered in infants and children. Functional obstructions (as seen in Hirschsprung's disease) and mechanical obstructions (such as jejunoileal atresia (JIA)) can produce dilatation of the small bowel [1].

Prolonged obstruction increases bowel distension and workload and can modify bowel motility. Little is known about the reversibility of smooth muscle changes after relief of obstruction. The animal experiments described here were undertaken in order to characterize the motility and histological alterations of smooth muscle and their potential reversibility following relief of obstruction. We discuss our results with respect to the scientific literature and the surgical management of ileal dilatation.

We addressed this issue by evaluating ileal motility in response to electrical and pharmacological stimulation. Intestinal motility is regulated by a number of neurotransmitters, which elicit either stimulatory or inhibitory effects on muscle contraction [2]. Lachykinin substance P and the neurotransmitter acetylcholine (ACh) are both involved in the excitatory neural pathways underlying peristaltic motor activity in the intestine. It has also been shown that nitric oxide (NO, synthesized from L-arginine by calcium-dependent, constitutive NO-synthase in neurons) mediates non-adrenergic, non-cholinergic (NANC) relaxation following neuronal stimulation in a wide range of intestinal smooth muscle [3]. In studies of strips of rat stomach and colon and human ileum and colon, incubation with NO-synthase inhibitors (such as N^G-nitro-L-arginine methyl ester (L-NAME)) increased the preparations' basal tension [4]. In vivo, intravenous administration of L-NAME caused a substantial increase in tone and spontaneous motility in the jejunum of the anaesthetized rat [5]. These latter effects thus suggest that endogenous NO has a role not only as a NANC neurotransmitter following stimulation but also in the

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regulation of resting motility in the small intestine (via interaction with other local mediators).

We therefore evaluated the contractility of the ileal smooth muscle by using electrical and pharmacological stimulation (KCl, ACh, substance P, L-NAME and histamine).

1. Materials and methods

1.1. Animals and surgical procedures

Twenty-one male Wistar rats were obtained from Janvier Laboratories (Le Genest St-Isle, France). The animals were housed under controlled conditions (temperature: 21 ± 1 °C; 12 h/12 h light/dark cycle) and had ad libitum access to food and water throughout the study. The experimental procedures were approved by the Animal Care and Use Committee at Jules Verne University of Picardy (Amiens, France). The mean ± SD weight for each group and each time point are presented in Table 1.

Surgical procedures were carried out under general anaesthesia after intraperitoneal injection of ketamine (0.3 mL/100 g body weight (bw)) and xylazine hydrochloride (Rompun, Bayer, Toronto, Canada (0.018 mL/100 g bw). A further half-dose was administered if the surgical procedure lasted more than 45 minutes. Postoperative analgesia was achieved via the intramuscular injection of 4% tolfenamic acid (Tolfedin CS from Vetoquinol, Lure, France) (0.01 mL/100 g bw/day)) the day after surgery. All the animals were monitored twice a day (in terms of pain, general well-being, scarring, food consumption and abdominal distension) until sacrifice by lethal anaesthesia at 8 weeks of age.

The bowel anastomosis was performed with microsurgical technique. The anastomosis and parietal wall closure were performed with absorbable suture (Vicryl 6.0 and Vicryl 4.0, respectively (Ethicon, Munich, Germany)).

The 21 rats were randomly assigned to three different groups: a control group, a sham group and an operated group (n = 7 in @ group). In the control group and the sham group, a 15-mm-long segment of bowel (designated as R1 and R2, respectively) was resected at 5 cm from the cecum in 8-week-old animals (at sacrifice). In the sham group, midline laparotomies were performed at 4 and 6 weeks; exteriorization of the cecum and the distal part of the small bowel was followed by parietal wall closure.

In 4-week-old animals in (operated) group 3, a narrow, isoperistaltic intestinal loop was created after a midline laparotomy. The ileum was sectioned (at 5 cm from the cecum) and closed at its distal end. Three centimetres upstream, the resected ileum was connected to the terminal ileum by a lateral-end anastomosis (Fig. 1a–d). Two weeks later, the same rats underwent a second midline laparotomy and a 15-mm-long distal fragment of the blind loop (designated as R3) was removed for analysis (Fig. 1e and f). The loop and the terminal ileum then underwent end-to-end anastomosis. Two weeks later, the animals were sacrificed and a 15-mm-long segment of ileum was removed upstream from the previous anastomosis (designated as R4) (Fig. 1g).

Table 1
The animals' body weight (in g): mean weight ± standard deviation for animals in the operated, sham and control groups.

	Age (wk)	4	6	8
Weight(g)	Operated	90 ± 8	218 ± 70	294 ± 13
	Sham	85 ± 7	317 ± 21	461 ± 46
	Control	87 ± 6	353 ± 41	524 ± 16

The mean weight in the operated group was somewhat lower than in sham group and markedly lower than in control group.

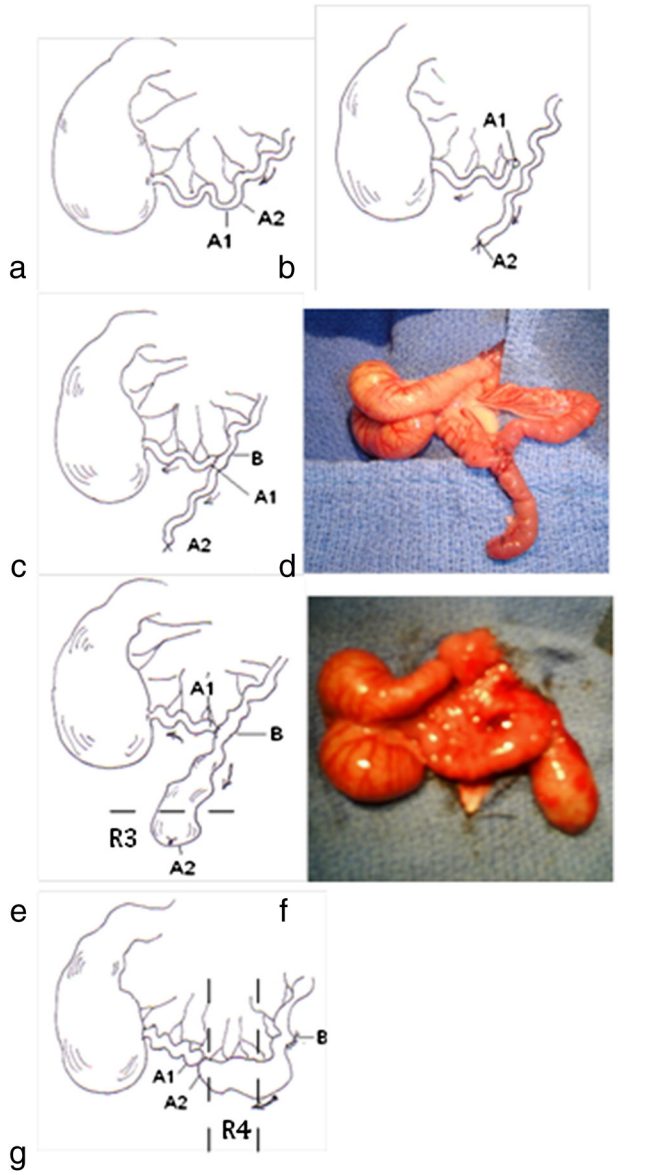

Fig. 1. Surgical procedures. (a–d): Creation of a blind loop (in 4-week-old animals): Section of the ileum between A1 and A2 (5 cm from the caecum), closure of the proximal end (A2) and creation of a lateral-end anastomosis with the terminal ileum (A1 and B). (e and f) The dilated loop: fragment R3 was removed from A2. (g) End-to-end anastomosis between the loop and the terminal ileum (A1-A2) and removal of a fragment from A2 (R4) (B: lateral closure).

The ileal segments R1 to R4 were assessed both in vitro motility and histological studies.

1.2. Organ bath analysis

1.2.1. Preparation of the muscle strips and the organ bath

Ileal segments (R1–R4) were placed in a Petri dish containing a physiological Krebs-Henseleit solution (composition: (g/L): D-glucose 2; $MgSO_4$ 0.141, KH_2PO_4 0.16, KCl 0.35, NaCl 6.9, $CaCl_2$ 3.73, $NaHCO_3$ 21, pH 7.4 (Sigma-Aldrich, Saint Louis, MO, USA)). This solution was also used to rinse the intraluminal contents out of the ileal segments. Circularly oriented muscle strips from ileum (10 × 5 mm) were mounted in tissue bath chambers (two strips per animal) containing 10 mL of warm (37 °C) Krebs solution saturated with 95% O_2 and 5% CO_2. The mechanical characteristics of the strips' smooth muscle were measured with an isometric force