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Effect of Tributyrin on Electrical Activity in the Small Intestine during Early Postoperative Period

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The effect of enteral administration of tributyrin on electrical activity in the upper segments of the small intestine was examined in rats on the model of postoperative ileus. This postoperative state is characterized with pronounced and long-term disturbances in generation of migrating myoelectric complex of the small intestine. The enteral administration of tributyrin in the early postoperative period aimed to suppress the non-adrenergic non-cholinergic influences and activation of the cholinergic anti-inflammatory pathways is an effective procedure to normalize the migrating myoelectric complex and therefore the coordinated propulsive peristalsis in the small intestine.

Key Words: *tributyrin; migrating myoelectric complex; small intestine; postoperative ileus*

It is a common view that the leading role in the blockade of coordinated propulsive peristalsis in the small intestine (SI) observed after abdominal surgical intervention is played by up-regulation of inhibitory influences mediated via activation of adrenergic, dopaminergic as well as non-adrenergic non-cholinergic influences, which employs NO as the major neurotransmitter.

Another factor playing no less important role in pathogenesis of motor postoperative disturbances is the inflammatory response to surgery aggression [2]. Even a simple manipulation with SI provokes transitory permeability elevation in mucous membrane accompanied by translocation of bacteria and toxins and local inflammatory response in the gut wall followed by accumulation of granulocytes at various stages of maturity in the muscular tunic resulting in pronounced dysfunction of SI muscles [9].

There are three hypotheses explaining the effect of proinflammatory neurotransmitters released from the intestine on its motility. They suggest that these neurotransmitters directly affect the smooth muscle cells, activate the neuronal inhibitory pathways in the enteral nervous system, and exert the global effect via activation of the higher motor centers in the brainstem resulting in inhibition of intestinal motility by CNS [4-6].

The popular prescription drugs that restore the motor function of SI are prokinetic agents. The last years advanced a novel approach in correcting the intestinal motor disturbances based on the use of remedies aimed to cope with inflammatory response. The major role in this approach is given to the short-chain fatty acids (SCFA) exemplified by butyrate, propionate, and acetate, which are important energy substrates in mucous tunic of small and large intestine. These fatty acids are produced during lysis of dietary fibers by the microorganisms through anaerobic fermentation.

Tributyrin is a structural lipid composed of three molecules of butyrate esterified with glycerol. In comparison with other sources of butyrate (dietary fibers and oils), tributyrin demonstrates an undisputable ad-

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vantage as a component of enteral nutrient mixture, because during critical conditions of the patients, the high doses of dietary fibers needed to produce SCFA can provoke abdominal distension, while a large amount of fat cannot be assimilated by such patients. Although few papers concern the physiological role of tributyrin, some studies showed that enteral administration of this agent down-regulates production of pro-inflammatory cytokines and NO [10].

To rectify this situation, we tried to assess the role of tributyrin in correcting the motor disturbances in SI provoked by surgical intervention into the abdominal organs.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats (n=12) weighing about 400 g. All animal protocols were approved by the local committee on biomedical ethics of N. V. Sklifosovsky Research Institute of Emergency Medicine.

After an 18-h food deprivation period, midline laparotomy was performed under ketamine narcosis. A probe was implanted into initial segment of SI (10 cm distally to ligament of Treitz). In addition, the needle electrodes were implanted into the wall of duodenum (DD) and into initial segment of SI (15 cm distally to ligament of Treitz).

The experiments were performed in the early postoperative period (days 1-6 after the surgery). During the first 3 days after the surgery, a single intraintestinal injection of 0.2 ml physiological solution or 0.2 ml tributyrin (10 mg/kg) was made in control (n=6) and experimental (n=6) rats, respectively. The electrical activity in examined gastrointestinal tract subdivisions was recorded by electromyography technique for 1 h prior to injection of tributyrin (baseline activity) and for 2 h after it. The intact control group comprised the rats (n=5) with implanted needle electrodes. In these rats, only electrical activity was recorded on postoperative day 10 after an 18-h food deprivation period.

On postoperative days 1 and 2, the rats were intraintestinally injected with glucose-salt solution (8 ml/400 g) after the end of recording period. Starting from day 3, the rats were given the standard diet. The free access to water and food was provided during entire postoperative period except for the time when the electrical activity was recorded. After experiments, the rats were sacrificed with a lethal dose of ketamine. Air temperature in the experimental room was 20-24°C. The rats were exposed to normal 12 h/12 h day/night cycle: light from 08.00 to 20.00, twilight from 20.00 to 08.00.

Electrical activity was recorded with an NVX-52 encephalograph within the frequency band of 0.1-30.0 Hz and processed in PC. To reveal spike activity, the signals were filtered in the band of 5-30 Hz. The electromyograms (EMG) were used to calculate the parameters of migrating myoelectric complex (MMC) viewed as the marker of normal coordinated propulsive peristalsis in the SI (MMC period, phase III duration, DD-SI propagation time for phase III).

The data were processed statistically with nonparametrical tests. The electrical activity parameters prior to and after tributyrin injection were analyzed with Wilcoxon's non-parametrical T test. The non-parametrical Mann–Whitney U test was employed to compare both control and experimental rats with the intact control animals. The results are presented with median and percentiles. Significance was assessed at $p<0.05$.

RESULTS

On the first postoperative day, EMG recorded in DD and SI in the control group revealed phase I (resting state) and phase II (irregular activity), while phase III

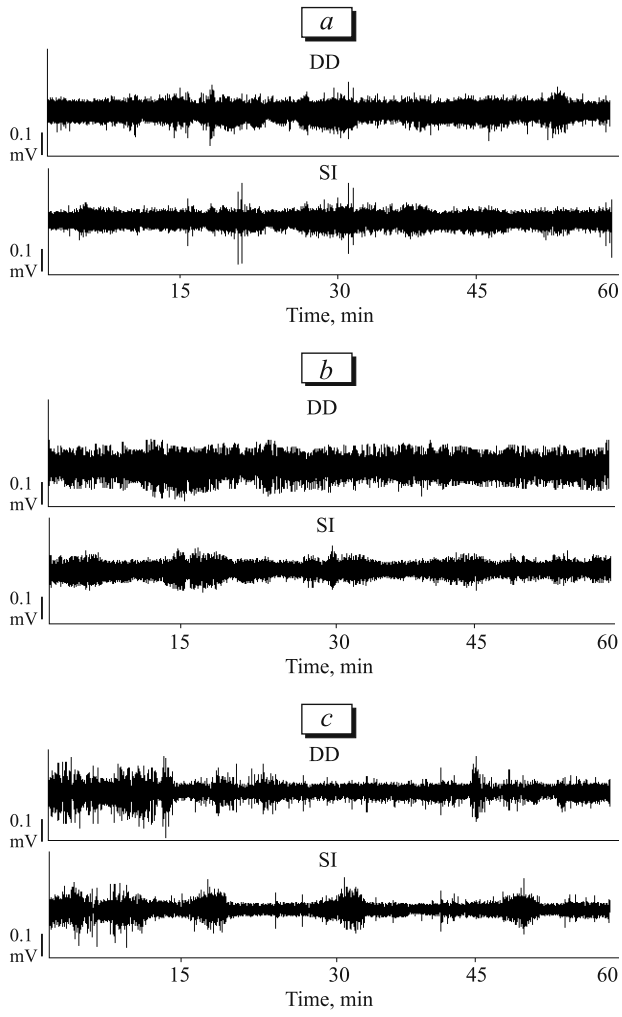

Fig. 1. The changes in electrical activity of DD and SI during the early postoperative period in the control rats on postoperative days 1 (*a*), 3 (*b*), and 6 (*c*).

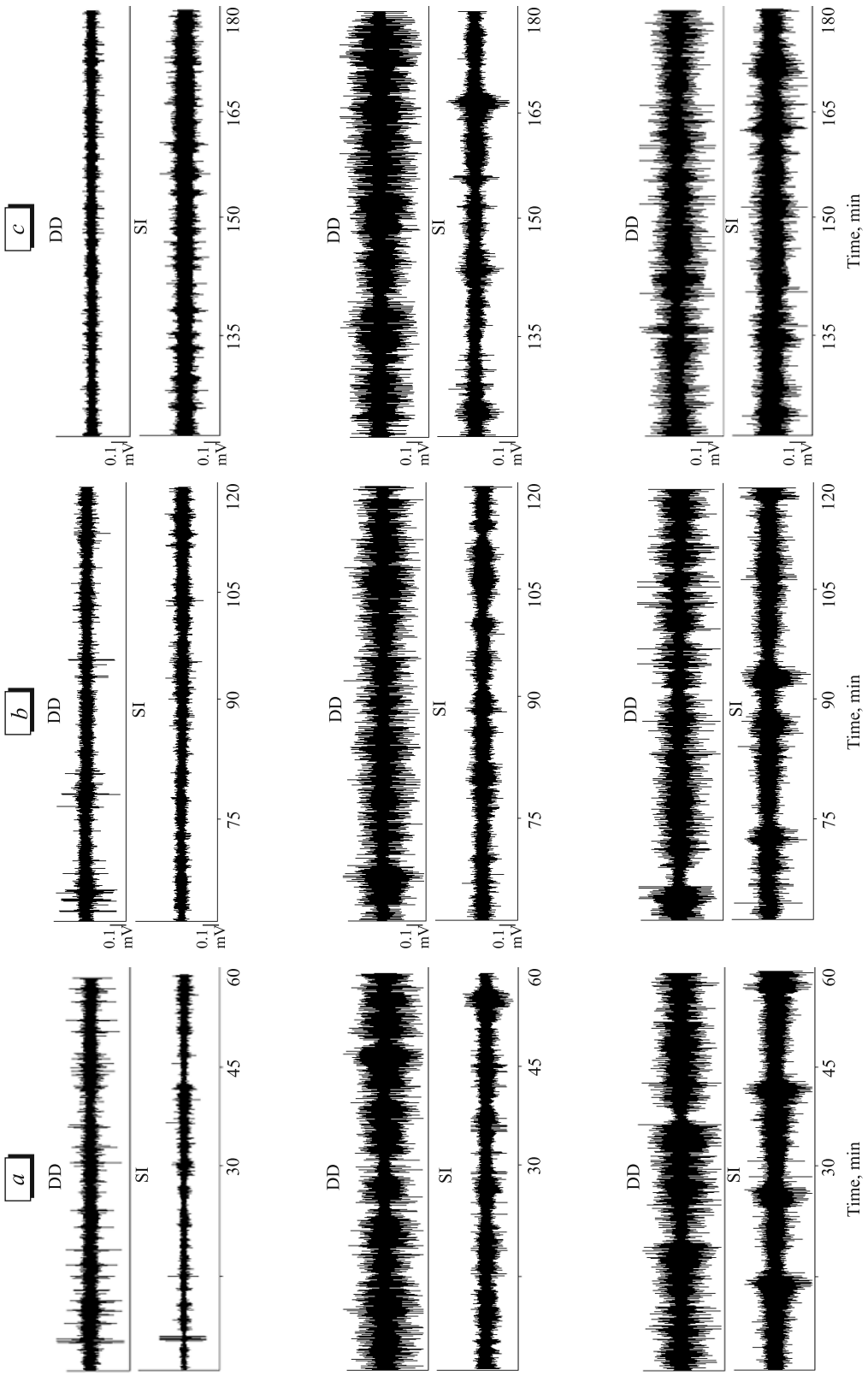

Fig. 2. Effect of tributyrin on electrical activity during the first 3 postoperative days. *a)* Baseline activity; *b-c)* after injection of tributyrin.

was absent (Fig. 1). Injection of physiological solution into SI evoked no significant electrical activity in DD and SI. On postoperative day 2, the baseline activity in both recorded areas did not change, although in some cases, SI demonstrated the phase III of electrical activity. On postoperative day 3, the baseline activity in most rats was characterized with all MMC phases observed both in DD and SI, although their durations and sequences were disturbed. The phase III propagation time of 120 (105; 125) sec was pronouncedly below the norm ($p<0.05$). To postoperative day 5, MMC parameters had been restored in SI, while in most rats, the phase III duration in DD was shorter than the normal value. To postoperative day 6, MMC parameters were restored in both examined areas of gastrointestinal tract, while the complex propagated from DD into SI. In DD and SI, MMC periods were 660 (533; 730) sec and 620 (560; 805) sec, respectively, the duration of phase III migration phase being 370 (250; 610) sec.

On the first postoperative day in experimental group, the baseline EMG records in DD and SI documented phase I (resting state) and phase II (irregular activity), while phase III was not revealed (Fig. 2). In most rats, injection of tributyrin induced phase III of normal duration in DD and SI: 160 (135; 165) sec and 145 (103; 190) sec ($p>0.05$), respectively. At this, migration of phase III activity was observed in half the rats. The migration time was 595 (508; 683) sec, which was far greater than the normal value ($p<0.05$). In one rat, we observed three successive MMC in DD, all of which propagated into SI.

On the second postoperative day, EMG in DD of most rats contained the phases II and III, but they had no resting phase. In contrast, EMG in SI demonstrated all the phases, but their succession and duration in MMC were abnormal. After injection of tributyrin, all three MMC phases were recorded in DD, and in half the rats, the phase III migrated into SI. At these rats, the migration period was 320 (315; 610) sec, which did not differ from the norm ($p>0.05$). In SI, MMC was characterized with normal succession of the phases and with the period of 675 (565; 810) sec, which did not differ from the norm ($p>0.05$).

On the third postoperative day, the baseline records in DD and SI of all rats revealed MMC. In DD, the duration of resting phase was below the norm, while the migration period of phase III did not differ from the control value being 370 (308; 435) sec ($p>0.05$). In SI, all MMC parameters corresponded to the norm: the period was 740 (700; 770) sec, and phase III duration was 180 (160; 215) sec ($p>0.05$). When injected on this day, tributyrin produced quite a different reaction in comparison with those observed on postoperative days 1 or 2. MMC disappeared both in DD ad SI. Moreover, on postinjection hour 2, the "feeding" motor activity with prevailing irregular bursts was observed, which was similar to that characteristic of the healthy rats.

Analysis of restoration of the electrical activity in SI during postoperative period in control and experimental groups showed that tributyrin produced a pronounced stimulating effect namely on propulsive motor activity by inducing phase III in MMC even on the first postoperative day resulting in complete restoration of MMC at the third day. Previously we established that after abdominal surgical interventions, suppression of inhibitory influences by injection of an inhibitor of NO synthesis restored MMC in SI [1]. It is also noteworthy that tributyrin down-regulates NO production [10]. In this study, tributyrin restored the phase III of MMC, which probably resulted from a decrease of NO level by this agent.

It should be remembered that tributyrin is a structural lipid, which interacts with the cholecystokinin receptors. It is a common knowledge that the lipids located in the intestine lumen activate autonomic nervous system via type 1 cholecystokinin receptors situated on vagus afferent terminals [3]. In its turn, vagal stimulation activated α7-nicotinic receptors of the intestinal macrophages thereby eliminating the inflammatory response in the intestinal wall via down-regulating the release of proinflammatory cytokines [7,8].

Thus, enteral administration of tributyrin in the early postoperative period aimed to suppress the non-adrenergic non-cholinergic influences and activate the cholinergic anti-inflammatory pathways is an effective therapy to normalize the coordinated propulsive peristalsis.

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