

Sodium Succinate Decahydrate Potentiates the Contractile Function of Duodenal Visceral Smooth Muscle in Male Albino Rat

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ABSTRACT

Sodium Succinate Decahydrate (SSD), a common food additive and acidulant, is recognized for its flavour enhancing property, buffering capacity and role in maintaining acid-base balance in processed and manufactured food stuffs. Despite its widespread use, its influence on the contractile function of the small intestinal visceral smooth muscle (SiVSM) has not been studied. This study investigates the effects of SSD on the contractile function of the SiVSM by examining its effect on the movement of isolated duodenal segments, the representative and crucial part of the small intestine in male albino rats. The isolated duodenal segments were exposed to graded concentrations of SSD in an organ bath, and changes in amplitude and frequency of contractions were recorded using an isotonic transducer IT- 2245 connected to RMS Polyrite-D. Our results showed that SSD significantly increases both the amplitude and frequency of duodenal contractions in a dose-dependent manner, indicating a stimulatory effect on the contractile function of the dVSM. The findings suggest that SSD may enhance the

contractile functions of the dVSM through potential modulation of the enteric nervous system probably by facilitating the activity of intrinsic cholinergic myenteric efferents and/or suppressing the activity of intrinsic adrenergic and/or nitrergic intrinsic myenteric efferents.

Keywords: Sodium succinate decahydrate, duodenal visceral smooth muscle, contractile function, enteric nervous system, intrinsic myenteric efferents.

INTRODUCTION

In the modern food industry, additives play a crucial role in enhancing flavor, extending shelf life, and improving product stability (European Food Safety Authority). Among these substances is sodium succinate, specifically in its decahydrate form (C₄H₄Na₂O₄·10H₂O), which is widely used as a flavoring agent and acidity regulator (E363) to impart a mild sour and salty taste, often described as contributing to the "umami" profile in savory foods (Ma et al., 2020). However, the use of any chemical compound in the food supply necessitates a thorough understanding of its safety and potential toxicological effects.

The seemingly contradictory roles of a substance being a food additive and a topic of toxicological study are reconciled by examining its biological context. In addition to its use as a food additive, succinate is a key molecule in the citric acid cycle (also known as the Krebs cycle), a metabolic process that generates energy. Within this cycle, the enzyme succinate dehydrogenase (SDH) converts succinate to fumarate. SDH is a crucial component of both the citric acid cycle and the electron transport chain in the mitochondria. SDH is a complex enzyme that uses a coenzyme called FAD to bind with substrates like succinate and fumarate, as well as with regulators like oxaloacetate and ATP. By oxidizing succinate, SDH helps connect a part of the citric acid cycle that rapidly breaks down acetyl-CoA. Succinate can be easily transported into the mitochondria using a specific carrier protein in exchange for another molecule, such as phosphate or malate. Mutations in the genes for SDH subunits are linked to various diseases, including Huntington's disease. Furthermore, succinate can act as an oncometabolite, a substance that promotes cancer development. It does this by inhibiting enzymes that remove methyl groups from histones and DNA. This inhibition leads to epigenetic silencing, which affects how cells develop and specialize, particularly in the neuroendocrine system (Pubchem).

The small intestine plays a crucial role in digestion and nutrient absorption, relying on the coordinated contractions of visceral smooth muscle (VSM) located within the muscularis externa layer to mix and propel luminal contents (Sanders et al., 2006). These motility patterns are regulated by a complex interplay of intrinsic myogenic mechanisms, interstitial cells of Cajal (ICC), and the enteric nervous system (ENS), which together generate and propagate rhythmic electrical activity known as slow waves or basal electrical rhythms (Takaki, 2003; Huizinga & Lammers, 2009). The ENS, composed of excitatory cholinergic

and inhibitory nitroergic or adrenergic neurons, modulates smooth muscle tone in response to various physiological and pharmacological stimuli (Furness, 2006; Grider, 2003).

In this context, the present study aims to investigate the *ex vivo* effect of sodium succinate decahydrate on the contractile functions of isolated duodenal segments from male albino rats. By analysing amplitude and frequency of contractions in response to graded SSD concentrations, this study seeks to elucidate the potential neuromodulatory role of SSD on duodenal smooth muscle, contributing to a broader understanding of how common food additives may influence gastrointestinal function.

MATERIALS & METHODS

Chemicals and Reagents

All the reagents and chemicals that were used to conduct this study were of analytical grade. The test chemical- sodium succinate decahydrate was procured from Sigma-Aldrich. Sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride (MgCl₂), calcium chloride (CaCl₂), sodium bicarbonate (NaHCO₃), sodium dihydrogen phosphate (NaH₂PO₄), glucose, etc. were procured from E. Merck, India.

Experimental Animals

As the experimental model, adult male albino rats of Sprague Dawley strain with body weight ranging around 130-150 g and age around 2-3 months were selected. They were kept in the room temperature of 25-27°C at the departmental animal care room with 24 hours light-dark cycle and were fed with laboratory chow and water and were kept in the animal house in accordance with the animal ethics committee's guidelines from Kalyani University.

Experimental Design

The animals were treated to different exposure conditions as mentioned in Table 1.

Table 1: Experimental Setup for the study

Groups	Exposure condition
Set 1	Application of 80 μ M SSD on the duodenal segments
Set 2	Application of graded doses of 160 μ M SSD on the duodenal segments
Set 3	Application of graded doses of 240 μ M SSD on the duodenal segments
Set-4	Application of graded doses of 320 μ M SSD on the duodenal segments

Animal Sacrifice

The selected animals were subjected to overnight fasting prior to sacrifice to standardize physiological conditions. Euthanasia was performed using cervical dislocation, ensuring minimal pain and distress, in strict accordance with the ethical guidelines approved by the Animal Ethics Committee of the University of Kalyani.

Collection of the Organ

Following cervical dislocation, the animal's abdominal cavity was opened. The small intestine was carefully dissected free from the mesentery, stomach, and large intestine via transverse incisions. The proximal portion of the small intestine, specifically the duodenum, was isolated for the study, as it exhibits the most prominent motility among intestinal segments. The collected duodenal segment was immediately transferred to a beaker containing temperature-controlled Tyrode's solution. The lumen was gently flushed to remove any residual contents. The cleaned segment was then promptly mounted in the organ bath of Dale's apparatus for *ex vivo* recording of spontaneous duodenal motility.

Recording of the Movement of the Duodenum

To record the spontaneous *ex vivo* motility of duodenal visceral smooth muscle (dVSM), a duodenal segment approximately 3 cm in length was vertically suspended in an organ bath containing 50 ml of Tyrode's solution. The segment was secured using two metal hooks inserted at both ends of the tissue. The composition of Tyrode's solution included: 8.0 g NaCl, 0.2 g KCl, 0.2 g CaCl₂, 0.1 g MgCl₂, 0.05 g NaH₂PO₄, 1.0 g NaHCO₃, and 1.0 g dextrose per liter, adjusted to pH 7.4. Oxygenation was maintained using a continuous flow of

oxygen at a rate of 2–3 bubbles per second, delivered directly into the organ bath via an oxygen bubbler. The bath temperature was kept at $37 \pm 0.5^\circ\text{C}$ using an automatic thermostat integrated with Dale's apparatus. The lower end of the duodenal tissue was anchored to the base of the organ bath, while the upper end was connected to the lever of an isotonic transducer (IT 2245). The transducer was interfaced with RMS Polyrite-D software (RMS, Chandigarh, India) to enable continuous recording of tissue contractions. Each tissue segment was allowed to stabilize for at least 35 minutes under these experimental conditions and was rinsed multiple times with fresh Tyrode's solution to remove metabolic residues. Isotonic contractions representing spontaneous rhythmic motility were recorded continuously following the administration of various concentrations of sodium succinate decahydrate (SSD).

STATISTICAL ANALYSIS

Data from each experimental group were expressed as mean \pm SEM. The frequency and amplitude of the recorded duodenal movements were analyzed to determine the contractile force. For functional assessments, the responses of treated tissues were calculated as percentage changes relative to their respective basal (control) values. Statistical comparisons among groups were performed using one-way ANOVA with GraphPad Prism 8 software. A *P* value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

In order to understand the effect of SSD on the contractile function of the dVSM, the movement of the duodenum in response to graded doses of SSD were recorded with the help of an isotonic transducer IT-2245

coupled to RMS Poyrite D. From the mechanical tracings, we have found that exposure to graded doses of sodium succinate decahydrate (SSD) on isolated duodenal segments in an organ bath setup

significantly enhanced both the amplitude and frequency of duodenal contractions in a dose-dependent manner (Figure 1 and Table 2).

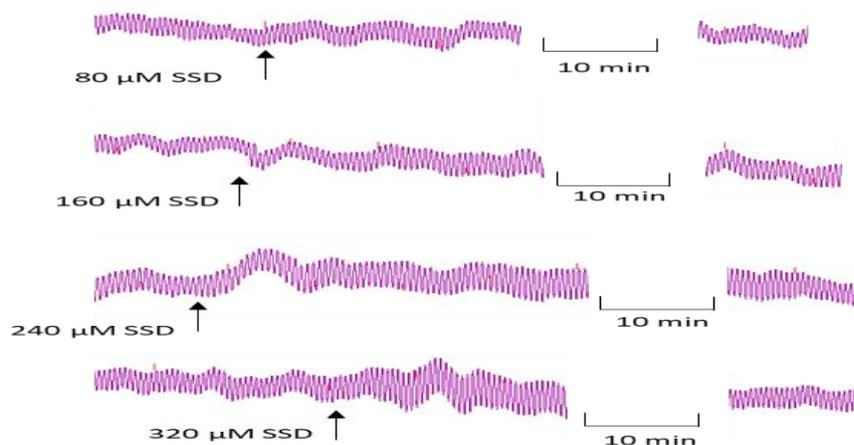


Figure 1. Tracings showing representative records of the effect of graded concentrations of Sodium succinate decahydrate (SSD) on the isolated duodenal segment in order to examine the effect of SSD on the movement of duodenum of rat in tissue organ bath obtained with an isotonic transducer coupled to RMS Polyrite-D.

This suggests that SSD may act directly on smooth muscle cells or indirectly via modulation of enteric neurotransmission of small intestine. The small intestine plays a vital role in digestion, absorption, secretion, and immune responses—functions that are largely dependent on the rhythmic contractions of visceral smooth muscles (VSM) located within the muscularis externa layer. These coordinated contractions ensure the proper mixing of luminal contents with pancreatic and biliary secretions and facilitate the aboral

propulsion of chyme, thereby promoting efficient digestion and nutrient absorption. The regulation of intestinal motility is primarily governed by the interstitial cells of Cajal (ICCs), which serve as pacemaker cells by generating and propagating electrical slow waves (ESWs) that determine the excitability of smooth muscle cells. ICCs also mediate the transmission of neural signals from the enteric nervous system (ENS), thereby integrating both myogenic and neurogenic mechanisms in the control of motility.

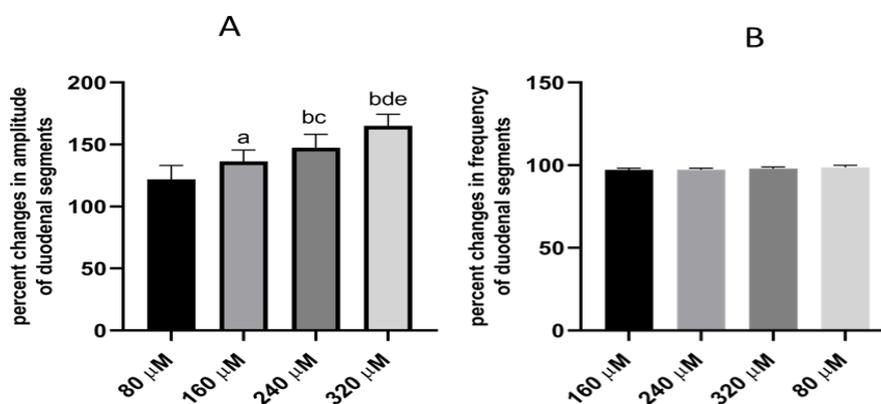


Figure 2. Bar diagrams showing the percent changes in the amplitude (A) and frequency (B) of contraction of the duodenum in SSD exposed groups (80 μM, 160 μM, 240 μM and 300 μM) compared to control. The data were represented as mean ± SEM for all the groups. ^{a,b}*p*<0.01, 0.0001 vs. 80 μM SSD, ^{c,d}*p*< 0.05, 0.0001 vs. 160 μM SSD and ^e*p*<0.0001 vs. 240 μM SSD (A).

SSD Concentration (mM)	Amplitude (% of control)	Frequency (% of control)
80 μ M	121.9 \pm 2.880	98.63 \pm 1.333*
160 μ M	136.4 \pm 2.344	97.20 \pm 1.024
240 μ M	147.5 \pm 2.781	97.26 \pm 0.8968
320 μ M	165.1 \pm 2.40	98.00 \pm 0.9759

Table 2. Table showing the percent alterations in amplitude and frequency of contractions of the dVSM as a result of exposure to SSD

In the current study, SSD exposure resulted in a dose-dependent enhancement of small intestinal visceral smooth muscle (SiVSM) contractile function under *ex vivo* conditions. (Figure 2 and Table 2). From

these findings it might be hypothesized that SSD may augment the generation and/or propagation of ESWs by modulating ICC activity that resulted in the increase in frequency of contraction of the dVSM.

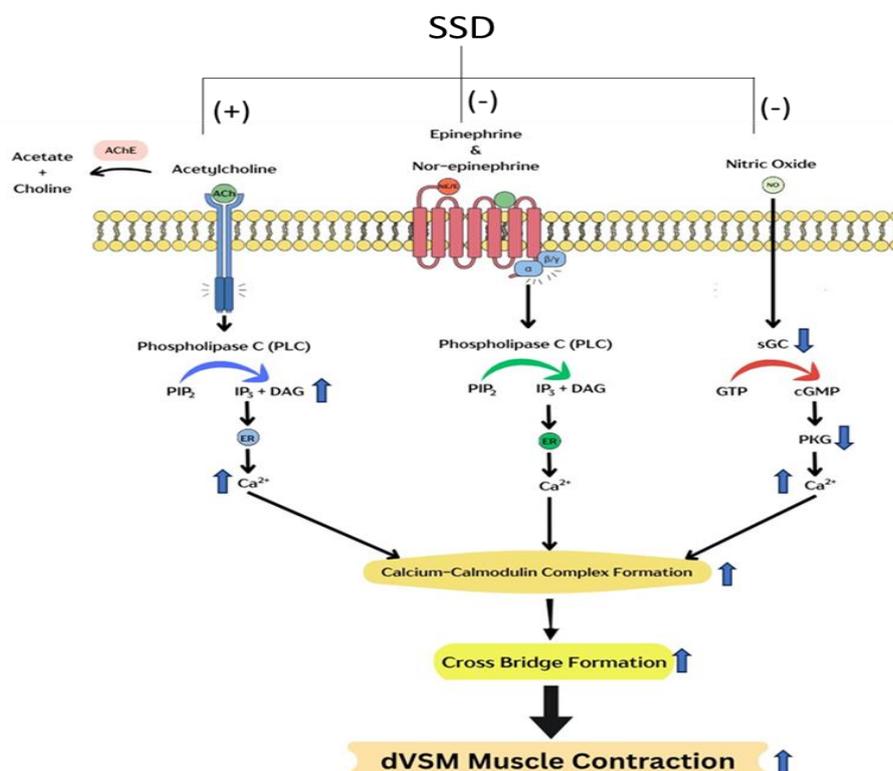


Figure 3. Schematic representation showing the probable intrinsic myenteric efferents involved in the SSD induced potentiation of the contraction of the dVSM. SSD- Sodium succinate hexahydrate; ACh-acetylcholine; NO-Nitric Oxide; NE/E- Norepinephrine/Epinephrine; AChE- Acetylcholinesterase; sGC- soluble guanylyl cyclase; $[Ca^{2+}]_i$ -Intracellular calcium concentration; Cal- Calmodulin; PIP₂ - Phosphatidylinositol 4,5-bisphosphate; IP₃- inositol 1,4,5-trisphosphate; cGMP- cyclic guanosine monophosphate; MLCK- Myosin light chain kinase. ↓ indicates decrease in activity or production; ↑ indicates increase in activity or production. +, indicates facilitation; -, indicates inhibition.

Additionally, SSD might influence the excitatory neural inputs from the ENS by altering neurotransmission pathways or disrupting membrane potential gradients of smooth muscle cells, thereby intensifying basal electrical rhythms (BERs). Moreover, the observed stimulatory effects of SSD on the amplitude of contractions of the dVSM might be due to the activation of excitatory

cholinergic myenteric efferents and/or the suppression of inhibitory signals mediated by adrenergic or nitrgenic/ NANC (non-adrenergic, non-cholinergic) neurons innervating the SiVSM (Figure 3). Further, sodium succinate, being an alkaline salt, may also influence calcium influx or membrane potential of smooth muscle cells

of intestine, promoting depolarization and contraction.

CONCLUSION

In conclusion, it could be suggested that sodium succinate decahydrate (SSD) markedly augments the contractile functions of small intestinal visceral smooth muscle (SiVSM) located within the muscularis externa layer of the rat small intestine by increasing amplitude and frequency of contractions of the dVSM. The SSD induced potentiation of the contractile function of the dVSM might be due to the activation of excitatory cholinergic myenteric efferents and/or the inhibition of inhibitory noradrenergic and non-adrenergic non-cholinergic (NANC) myenteric pathways innervating the SiVSM. Prolonged or repeated exposure to SSD-contaminated food could therefore lead to increase intestinal motility, potentially predisposing individuals to gastrointestinal disturbances or motility-related disorders.

Declaration by Authors

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