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Potentiation of the Contraction of the Duodenal Visceral Smooth Muscle by Potassium Bromate ($KBrO_3$) through Facilitation of Intrinsic Cholinergic Efferents

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ABSTRACT

In recent times, intoxication from chemicals used as food additive and adulterants poses a significant environmental health concern. Potassium Bromate commonly used as dough improver in making bread and other flour related products in an unrestricted quantity. Potassium Bromate ($KBrO_3$) primarily affects the small intestine through consumption of $KBrO_3$ contaminated foods. However, the toxic impact of $KBrO_3$ on duodenal visceral smooth muscle motor activity has not been studied. Therefore, the study aimed to investigate the potential toxic effects of $KBrO_3$ on the contraction of duodenal visceral smooth muscle ex vivo in a rat model. The findings show that when the duodenal segment was exposed to $KBrO_3$ in Dale's organ bath in an *ex vivo* experiment, the movement of the duodenum was greatly increased in a dose-dependent manner. Furthermore, we have demonstrated that applying $KBrO_3$ in combination with acetylcholine can synergistically potentiate the amplitude of the duodenal contraction. Additionally, it was noted that when the doses of $KBrO_3$ were administered to the atropine pre-incubated duodenal preparation in organ bath, the $KBrO_3$ induced potentiation of the contraction of the dVSM has been restricted. Therefore, from the findings, it can be concluded that $KBrO_3$ potentiates the contractile activity of the dVSM by facilitating the contractions of the smooth muscle located at the wall structure of the duodenum, most likely by enhancing the activity of intrinsic myenteric cholinergic efferents.

Keywords: Potassium bromate, motor activity, duodenal visceral smooth muscle, intrinsic myenteric efferents

INTRODUCTION

Potassium Bromate ($KBrO_3$) is used as an oxidising agent in food industry. It is extensively used in bread making as an agent of maturation (Ahamad et al., 2016). Due to its cheapness, it is the best choice for dough improvers in the baking industry. $KBrO_3$ has a characteristic role in food bio molecules-such as starch and protein as it regulates the external gelatinization, viscosity, swelling characteristics as well as gluten proteins (Ojeka et al 2006). Removal of the sulphydryl groups leads to the formation of disulphide linkages improving the properties of the bread (Shanmugavel et al 2019). It can even improve the quality of low quality gluten. Despite of being such use of it, it has been considered as carcinogenic (IARC, 1986), genotoxic (Kaya and Topaktaş (2007)), mutagenic (Ishidate et al., 1984), cytotoxic (Zhang et

al. (2010)) and also possess reproductive toxicity (Fadul et al., 2016). Due to its probable toxic threats, FAO/WHO experts restricted the use of $KBrO_3$ as an additive to flour (WHO,1992). Further, FDA has standardised that the acceptable levels should be lower than 75mg/kg or 50 mg/kg (FDA,2006).

Small intestinal motility is the ability of the smooth muscles of the small intestine (duodenum) to contract. Regulation of duodenal motility is the end result of intrinsic and extrinsic paracrine and neural regulation (David et al, 2013). The small intestinal motility helps in digestion and absorption by mixing the intestinal contents with enzymes. Through consumption of food stuffs, the small intestine gets immediately exposed to a variety of nutrients, chemicals, and/or antimicrobial agents. In addition to its roles in digestion and absorption, the small intestine guards the GI tracts and serves as a sentinel for mucosal immunity. Long-term ingestion of food additives can lead to the development of metabolic syndrome, colon cancer, and other forms of colitis.

Thus, any impairment in the contractions of the visceral smooth muscle located in the wall structure of the small intestine in response to any environmental toxicants or toxins will definitely hamper the digestive and absorptive functions of the gastrointestinal system that will lead to onset of different gastrointestinal disorders.

It is believed that $KBrO_3$ may cause toxicity to the contractile mechanism of visceral smooth muscle, which contributes the small intestine motility in humans exposed to $KBrO_3$.There hasn't been any research on the potential harmful effects of $KBrO_3$ on the contractile functions of small intestinal visceral smooth muscle till to date. Therefore, the aim of this study was to investigate how $KBrO_3$ affected the contractile function of visceral smooth muscle (VSM), which is located in the wall structure of the duodenum, a representative portion of the small intestine that aids in food absorption and digestion by giving the duodenum motility.

MATERIALS & METHODS

Chemicals and Reagents

All the chemicals including reagents and solvents used to fulfil this study here of analytical grade. Potassium bromate ($KBrO_3$), Atropin sulphate, acetylcholine chloride was purchased from sigma Aldrich, USA. sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), sodium dihydrogen phosphate (NaH_2PO_4), glucose, etc. were procured from E.Merck, India.

Animals

This investigation was carried out on mature albinorats weighing between 130 and 150 grams. The animals were housed in the departmental animal house, which had a typical 12-hour light-dark cycle and a temperature range of 25 to 27 degrees Celsius. They were fed a regular laboratory diet consisting of water and laboratory chow. The University's institutional animal ethics committee's recommended criteria were followed for conducting studies on animals.

Experimental Design

The animals were exposed to different doses and exposure conditions as mentioned in Table 1.

Table 1. Experimental design for the study

Groups	Exposure conditions
Set 1	Application of graded doses of $KBrO_3$ (5, 10, 20, 40 µM) on the duodenal segments
Set 2	Application of single dose of ACh (0.01 µM) on the duodenal segments
Set 3	Application of effective dose of $KBrO_3$ (20 µM) on duodenal segments pretreated with ACh (0.01 µM)

Table no 1 continued…	
Set 4	Application of single dose of Atropine (1 µM) on the duodenal segments
Set 5	Application of graded doses of $KBrO_3$ (20 and 40 µM) on duodenal segments pretreated with Atropine (1 µM)

Recording of the Duodenal Motility

To study the intestinal motility the rats are sacrificed by cervical dislocation after overnight fasting conditions, the abdomen was immediately opened and about 3cm long duodenum segment was cut by a scissors .Then it is cleaned by Tyrode's solution and the outer fat layer was removed because it may prevent the motility of the intestine .Then the segment was positioned vertically with the help of two metal hooks piercing through the two opposite ends of the tissue segment and was immersed into an organ bath filled with Tyrode's solution (NaCl-8g, KCl-0.2g, $CaCl_2$-0.2g, $MgCl_2$-0.1g, NaH_2PO_4-0.05g, $NaHCO_3$-1g, glucose -1g in1000 ml distilled water). After placing the tissue in the organ bath proper oxygen supply was provided and the temperature was maintained at 37ºCby using automatic thermostat machine attached with the Dales fluid bath. Following this, the movement of the duodenal segment was recorded by an isotonic transducer (IT-2245) coupled to RMS polyrite –D (RMS,Chandigarh ,India). Tyrodes solution was used to perform lengthy washes on the preparations with the purpose of eliminating accumulated metabolites while they were equilibrated for a minimum of forty-five minutes (Sarkar et al 2013).

Statistical Analysis

The data values for every experimental group were presented as means ± standard error of mean. The force of contractions was computed using the frequency and amplitude of the movement records. The percentage change from the basal (or control) values represented the values of the treated preparations for the functional tests.

To evaluate any significant differences between the groups, one-way ANOVA was performed (GraphPad Prism 8). $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Effect of Potassium Bromate on the Contractile Activity of the dVSM *Ex Vivo* of Rat

Graded doses of $KBrO_3$ were administered in single dose acute experiments, and the movement of the duodenum (*ex vivo*) was observed in order to investigate the effect of $KBrO_3$on the contractile activity of the dVSM. The tracings indicate that the isolated duodenal segments exhibited dose-dependent increase in amplitude upon exposure to graded doses of $KBrO_3$. Additionally, there was a dose-dependent decrease in the frequency of the dVSM contractions upon exposure to $KBrO_3$ (Figure 1).

The findings imply that $KBrO_3$potentiates the dVSM's contractile activity by increasing the amplitude and frequency of the smooth muscle's contractions, found in the duodenum's wall structure. The intrinsic myenteric efferents innervating the dVSM regulate the contractions of the visceral smooth muscle of the GI system which are primarily excitatory cholinergic, inhibitory adrenergic, and inhibitory nitrergic (NANC, non-adrenergic non cholinergic) myenteric efferents. Therefore, it is anticipated that the augmentation/activation of cholinergic myeneteric efferents and/or inhibition of adrenergic/nitrergic (NANC) intrinsic myenteric efferents innervating the dVSM may be the cause of the $KBrO_3$-induced potentiation of the contractile activity of the dVSM. Further, the increase in the

frequency of contraction of the dVSM might be due to increase in the rhythmicity of slow waves or Basal electrical rhythm (BERs) of the smooth muscles.

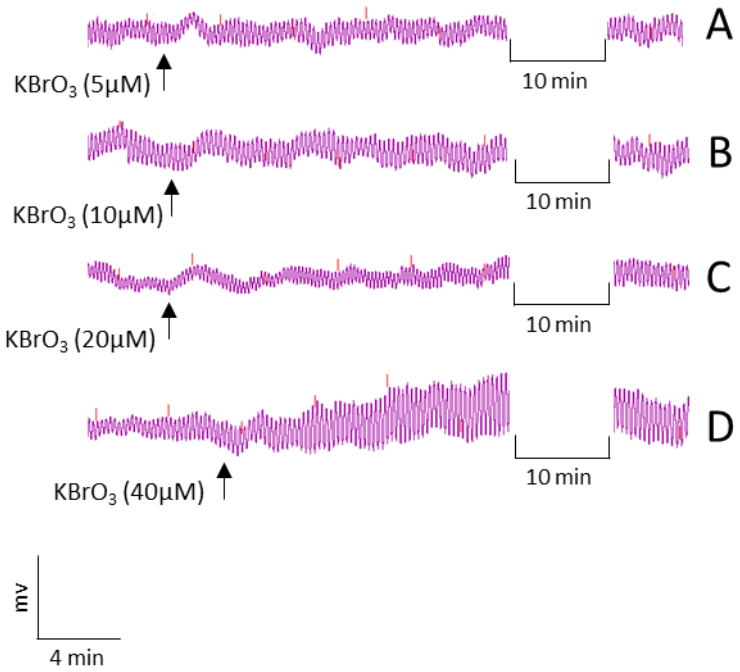

Figure 1. Tracings showing representative records of the effect of graded concentrations of $KBrO_3$ on the isolated duodenal movement of rat in tissue organ bath obtained with an isotonic transducer coupled to RMS Polyrite-D.

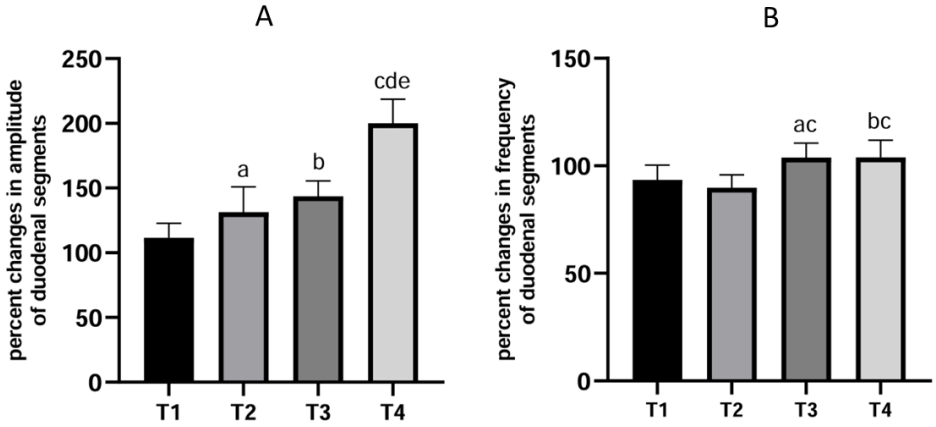

Figure 2. Bar diagram showing percent changes in amplitude and frequency of the contractions of isolated duodenum in response to the application of graded doses of $KBrO_3$. The data represented were mean ± SEM for all the group. $^{a,b,c}P$<0.05, 0.001, 0.0001 Vs T1; ^{d}P< 0.0001 Vs T2; ^{e}P <0.0001 Vs T3 (A). $^{a,b}P$<0.05, 0.001 Vs T1; ^{c}P< 0.01 Vs T2(B).

Effect of Potassium Bromate and Acetylcholine in Combination on the Contractile Activity of the dVSM *Ex Vivo* of Rat

The involvement of cholinergic intrinsic myenteric efferents was investigated in order to examine the probable pharmacodynamics involved in the $KBrO_3$ induced potentiation of the contractile

activity of the dVSM. The intrinsic cholinergic myenteric efferents are primarily excitatory to the contractions of the visceral smooth muscle located at the duodenal wall, as they release the excitatory neurotransmitter acetylcholine, which promotes the contraction of the visceral smooth muscle.

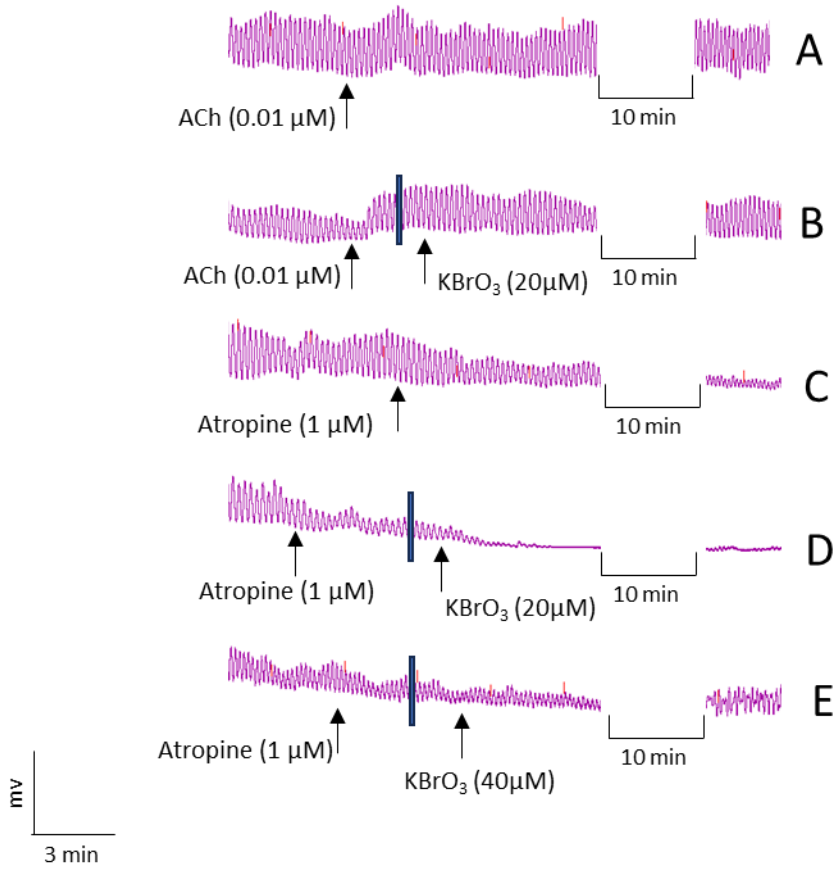

Figure 3. Tracings showing representative records of the effects of $KBrO_3$on the movement of duodenum in ACh and Atropine pre-treated duodenal preparations *ex vivo*. A: Tracing of effect of ACh (0.01 µM) on the movement of duodenum. B: Tracing of the effect of $KBrO_3$ (20 µM) on the movement of duodenum in ACh (0.01 µM) pre-treated duodenal preparations. C: Tracing of the effect of atropine (1 µM) on the movement of duodenum. D: Tracing of the effect of $KBrO_3$ (20 µM) on the movement of duodenum in atropine (1 µM) pre-treated duodenal preparations. E:Tracing of the effect of $KBrO_3$ (40 µM) on the movement of duodenum in atropine (1 µM) pre-treated duodenal preparationsobtained with an isotonic transducer coupled to RMS Polyrite-D.

Thus, the movement of the duodenum *ex vivo* in a single dose acute experiment was recorded in response to the application of $KBrO_3$ and acetylcholine, a cholinergic agonist, in order to determine the cholinergic myenteric influences in the $KBrO_3$ induced potentiation of the contractile activity of the dVSM. The tracings (Figure 3) indicate that pre-treatment of duodenal segments with ACh increased the degree of potentiation of the dVSM contraction in comparison to $KBrO_3$ alone. The synergistic potentiation of $KBrO_3$ and ACh together suggests that cholinergic myenteric efferents may have been activated, which in turn augmentsthe contractile activity of the dVSM.

Effect of Potassium Bromate and Atropine in combination on the contractile activity of the dVSM *Ex Vivo* of Rat

The movement of the duodenum *ex vivo* in a single dose acute experiment was recorded in response to the application of $KBrO_3$ and atropine, a cholinergic antagonist (cholinergic receptor blocker), in order to determine the activation of cholinergic myenteric efferents in the $KBrO_3$ induced potentiation of the contractile activity of the dVSM. The tracings showed that while atropine alone initially inhibits the dVSM contractions, this inhibitory action does not last very long. In contrast, the application of $KBrO_3$ in combination with atropine counteracted the $KBrO_3$-induced potentiation of the dVSM contraction, as observed in comparison to the potentiation of the dVSM contraction exhibited by $KBrO_3$ alone.

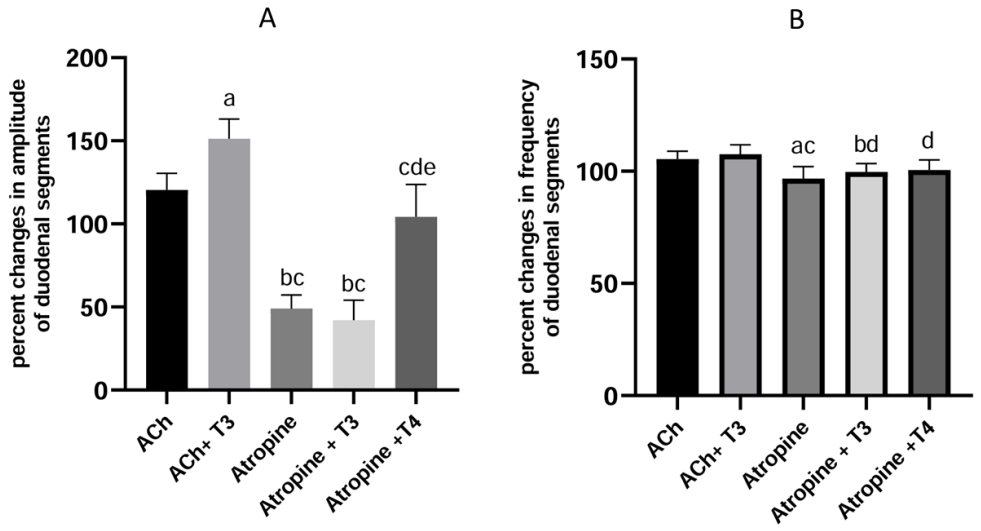

Figure 4. Bar diagram showing percent changes in amplitude and frequency of the contractions of isolated duodenum in response to the application of $KBrO_3$in combination with cholinergic agonist and antagonist(s). The data represented were mean ± SEM for all the group. $^{a,b}P$<0.001, 0.0001 Vs ACh; $^{c,}P$< 0.0001Vs ACh+T3; ^{d}P <0.0001 Vs Atropine; ^{e}P < 0.0001 Vs Atropine+T3 (A). $^{a,b}P$<0.001, 0.05 Vs ACh; $^{c,d}P$<0.0001, 0.01 Vs ACh+T3(B).

From the tracings (figure 3), it was observed that the lower dose even failed to facilitate the contractile activity of the dVSM in presence of atropine while the highest dose applied in this study slightly able to exhibit its facilitatory effect of $KBrO_3$ in presence of atropine. Even the facilitatory effect of the highest dose of $KBrO_3$ has been counteracted in presence of Atropine. This clearly suggests that as the cholinergic receptors were blocked by the atropine, $KBrO_3$ failed to exhibit its facilitatory action on the contractile activity of the dVSM. This clearly suggested that the $KBrO_3$ induced potentiation of the contractile activity of the dVSM is due to the augmentation of the activity of intrinsic myenteric cholinergic efferents that promotes the release of excitatory Acetylcholine and results in potentiation of the contraction of the dVSM (Figure 5).

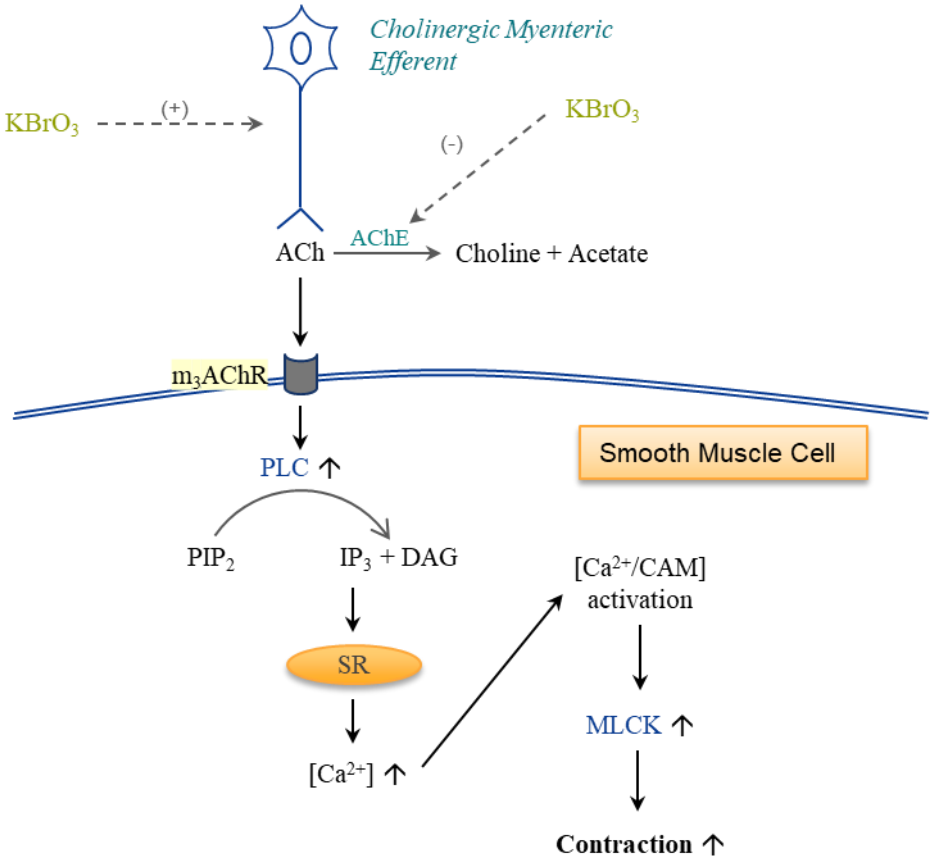

Figure 5. Schematic representation of the probable neurocrine mechanisms involved in the $KBrO_3$ induced potentiation of the contractile activity of the dVSM. (+); indicates stimulation, ↑indicates increase in levels.

CONCLUSION

$KBrO_3$ is extensively used in food industry as a dough improver. $KBrO_3$ impairs the contractile activity of the dVSM most likely by potentiating the contractions of the visceral smooth muscle located in the wall of duodenum. $KBrO_3$ potentiates the contraction of the dVSM by increasing the activity of cholinergic myenteric efferents presumably by promoting the release of acetylcholine and blocking AChE activity at myoneural junctions. The $KBrO_3$ induced impairment in the contractile activity of the dVSM that provides motility to it might results in impaired digestive and absorptive functions. The results from this study could be extrapolated in humans and it can be assumed that chronic exposure to $KBrO_3$ could result in impaired digestive and absorptive functions.

Declaration by Authors

Ethical Approval: Approved

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Conflict of Interest: The authors declare no conflict of interest.

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