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Original Article

Clostridium Difficile Toxins Impact on Rat Colon Smooth Muscle Reactivity

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Abstract

Aim: The present study was conducted in an attempt to find possible direct mechanisms of action of *Clostridium difficile* toxins A and B (TCdA and TCdB) on contractility of isolated rat intestinal smooth muscles, as the contractive pathways affected by the toxins and responsible for motility disorders remain unclear.

Materials and methods: Adult male Wistar rats were used in our experiments. Longitudinal smooth muscle (SM) preparations of proximal colon were isolated and their contractile activity was isometrically registered. The samples were mounted in tissue baths and exogenously treated with acetylcholine (ACh), serotonin (5-HT), dopamine, norepinephrine, TCdA and TCdB. The potential of TCdA and TCdB to affect the action of these mediators on SM activity was examined.

Results: The experiments have shown that exciting action of ACh and 5-HT on colonic contractility is enhanced by TCdA rather than TCdB. Conversely, relaxing effect of dopamine and norepinephrine on contractile activity of colonic SM is under impact of TCdB but not TcdA. TCdA has a stronger direct effect on *in vitro* SM sensitivity to ACh and 5-HT than TCdB.

Conclusions: TCdA and TCdB affect directly the contractile reactivity of isolated rat colon smooth muscle. TCdA has a stronger direct effect on smooth muscle sensitivity to acetylcholine and 5-HT than TCdB. Such a trend has not been established for dopamine and norepinephrine.

Keywords

Clostridium difficile, rat, smooth muscle

INTRODUCTION

The predominant sample of published experimental data concerning in vitro studies on *Clostridium difficile*-targeted tissues offers unclear hypotheses about the effect of TCdA and TCdB on SM contractile apparatus. Numerous studies apply an in vitro approach to elucidate the mechanisms of action of TCdA and TCdB. Conceptual similarities with the present work are found in projects that impose current ideas about changes in SM activity under the influence of toxins produced by *Clostridium difficile*. Ex vivo experiments with isolated intestinal muscle strips[1] have shown changes in contractile activity after treatment with TCdA and TCdB.

Under such conditions, the autogenous regulation of contractility (the stimulated contraction in areas with muscle wall overstretching) is disrupted. It reflects on the superposition of myogenically generated peristaltic rhythms.

As expected, a consequence of this is the impaired intestinal resorption. Such contractile changes of the colon lead to impaired water metabolism and, accordingly, to impaired formation of fecal masses and change in evacuation.[2]

In such contractile disorders, it should be of some interest to find out to what extent the intramural neuronal regulation[3] is able to compensate for them. We conducted ex vivo experiments using some major mediators modulating tract functions. The concept of the experiments included taking into account possible changes in the reactivity of SM isolated from rat colon and treated with TCdA and TCdB. The dominant modulator of the bioelectrical and contractile activity of the gastrointestinal muscles is ACh[4], released from the parasympathetic part of the autonomic splanchnic innervation. Some authors[5] point to the cholinergic system as predominantly influenced by the action of TCdA and TCdB.

AIM

The aim of the study was to clear up possible direct mechanisms of action of TCdA and TCdB on contractility of isolated rat intestinal smooth muscles.

MATERIALS AND METHODS

We used 38 adult male Wistar rats weighing 250–320 g in the experiments. The requirements set out in Directive 86/609/EEC for accommodation and care of experimental animals were fully met. The animals were housed under standard living conditions: temperature 23±1°C, change of light and dark – 12/12 hours, relative humidity about 45%. Approximately 12 hours before the start of experiments, the rats were separated from food.

We obtained permission for the study from the Bulgarian Food Safety Agency: Permission No. 116 for the use of animals in experiments. The experimental work was approved by the Commission for Scientific Ethics at the Medical University of Plovdiv with protocol No. 2/13.06.2019.

Smooth muscle preparations

SM preparations were excised in situ, separating the muscle tissue without mucosa as follows: a section with a length of about 3-4 cm was previously isolated from a proximal colon (2.5 cm after caecum). The longitudinal preparations of the colon used to record the contractile activity were 15-18 mm long and 1.0-1.1 mm wide. The circular intestinal strips had a length equal to the circumference of the intestinal wall in the respective region of the tract and a width of 1.0-1.2 mm. During dissection, SM tissues were washed with a preparation solution containing NaCl/ KCl/$CaCl_2$ in the ratio of 27.2:1.1:1.

During the experiment, the SM preparations were fixed in a tissue bath with Krebs solution (pH=7.4, t°=37°C) with the following content (mmol/L): NaCl – 120; KCl – 5.9; $CaCl_2$ – 2.5; $MgCl_2$ – 1.2; NaH_2PO_4 – 1.2; NaHCO – 15.4, and glucose – 11.5. All chemicals used to make the solution are manufactured by Merck. The pH of the solution was measured using a pH meter (HANNA). Krebs solution in contact with the SM preparations was aerated continuously during the experiment with a gas mixture O_2/CO_2 in the ratio of 19/1 (v/v).

Treatment of muscle preparations

Isolated SM were exogenously treated with ACh, 5-HT, dopamine, and norepinephrine by adding a precisely defined volume of concentrated solution of the respective substance necessary to achieve the desired concentration in the tissue bath (1.10^{-6} mol/L). The volume did not exceed 1/100 of the volume of the solution in the tissue bath. The vitality of SM tissue was tested by exposure to 1.10^{-6} mol/L ACh at the beginning of each experiment twice, after adaptation period of 60 minutes.

Assay of mechanical activity of smooth muscle preparations

Mechanical activity was recorded isometrically quantifying the contractile reactions in mN. The SM preparations were fixed to a glass holder at one end and to Swema strain gauges (Stockholm, Sweden) at the other by surgical sutures, with the gauges converting the mechanical deformation produced by contractile activity into a proportional electrical signal.

The initial mechanical stress for the preparations achieved by tensioning had a value corresponding to a force of 10 mN. The adaptation period to establish a baseline level of tone and regular spontaneous contractile activity was 60 minutes during which time the Krebs solution was changed two or three times. Changes in spontaneous mechanical activity and tone caused by exposure to various substances were reported relative to the corresponding baseline. Strength and frequency of phasic activity were defined as the mean of a large number of consecutive contractions (about 10).

The electrical signal from the strain gauges was amplified by K. Tesar-D 486 (Germany). The recording of mechanical activity on paper tape was performed using a Linseis recorder (Selb, Germany).

Assay of smooth muscle tissue response to *Clostridium difficile* toxins

Pre-dissolved in distilled water, the toxins were added to the Krebs solution (at standard conditions) in the tissue bath at the appropriate volume ratio to achieve the required concentration of TcdA – 1.10^{-8} mol/L and TCdB – 1.10^{-8} mol/L. After that, the incubation lasted 3 hours, and the incubation medium was replaced with fresh Krebs solution.

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Presentation of results and statistical processing

The results obtained from the experiments were statistically analyzed using STATISTICA 12.0 (StatSoft, Tulsa, Oklahoma). The analysis was performed on groups of independent or dependent variables determined by the type of experiment. The final values were presented as mean ± standard error. The value of Student's coefficient $p<0.05$ is accepted as criterion for significant difference.

RESULTS

Effects of ACh (1.10^{-6} mol/L) on the contractile activity of isolated smooth muscle preparations from colon circulum and colon longitudinalis.

Colon circulum

In control preparations, administration of ACh elicited a contractile response of 2.04±0.34 mN (n=20) without significant changes in the amplitude of the phase contractions (n-number of SM strips used in a given group) **(Fig. 1)**.

Incubated with 1.10^{-8} mol/L TCdA SM preparations (n=18) responded to exogenously administered ACh with a contraction of 2.63±0.44 mN, significantly increased compared to the control ($p<0.05$), without changes in the amplitude of phase contractions **(Fig. 1)**.

Incubated with 1.10^{-8} mol/L TCdB SM preparations (n=10) responded to exogenously administered ACh with a contraction of 2.01±0.71 mN without changes in the amplitude of phase contractions **(Fig. 1)**.

Colon longitudinalis

In the control preparations, the application of acetylcholine elicited a contractile response of 3.71±0.8 mN (n=20) without significant changes in the amplitude of phase contractions **(Fig. 1)**.

The SM preparations (n=15) incubated with 1.10^{-8} mol/L TCdA responded to exogenously administered ACh with a contraction of 5.96±0.7 mN significantly increased compared to the control ($p<0.05$) without changes in the amplitude of phase contractions **(Fig. 1)**.

The SM preparations (n=10) incubated with 1.10^{-8} mol/L TCdB responded to exogenously administered ACh with a contraction of 3.59±0.71 mN without changes in the amplitude of phase contractions **(Fig. 1)**.

Effects of 5-HT (1.10^{-6} mol/L) on the contractile activity of isolated smooth muscle preparations from colon circulum and colon longitudinalis.

Colon circulum

In control preparations, administration of 5-HT elicited a contractile response of 2.41±0.436 mN (n=20) without significant changes in the amplitude of phase contractions **(Fig. 2)**.

The SM preparations (n=18) incubated with 1.10^{-8} mol/L TCdA responded to exogenously administered 5-HT with a contraction of 1.25±0.64 mN without changes in the amplitude of phase contractions **(Fig. 2)**.

The SM preparations (n=10) incubated with 1.10^{-8} mol/L TCdB responded to exogenously administered 5-HT with a contraction of 2.02±0.92 mN without changes in the amplitude of phase reductions **(Fig. 2)**.

Colon longitudinalis

In control preparations, administration of 5-HT elicited a contractile response of 1.89±0.46 mN (n=20) without significant changes in the amplitude of phase contractions **(Fig. 2)**.

The SM preparations (n=15) incubated with 1.10^{-8} mol/LTCdA responded to exogenously administered 5-HT with

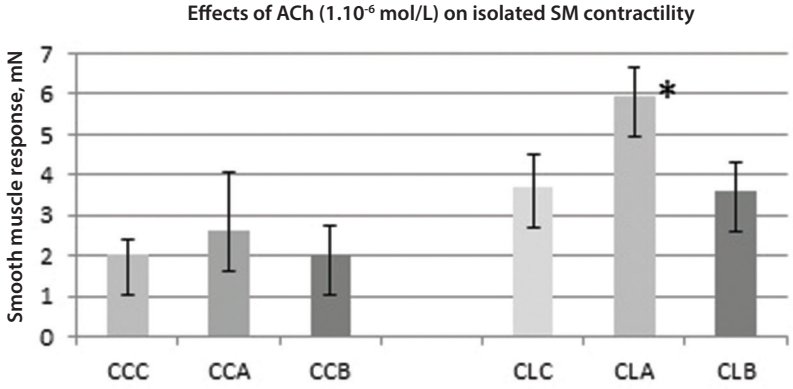

Figure 1. Contractile responses of isolated SM preparations to exogenously administered ACh (1.10^{-6} mol/L). CCC: circular column – control; CCA: circular column incubated with TCdA; CCB: circular column incubated with TCdB; CLC: longitudinal column – control; CLA: longitudinal column incubated with TCdA; CLB: longitudinal column with TCdB. Significant differences compared to the control responses were indicated by * ($p<0.05$).

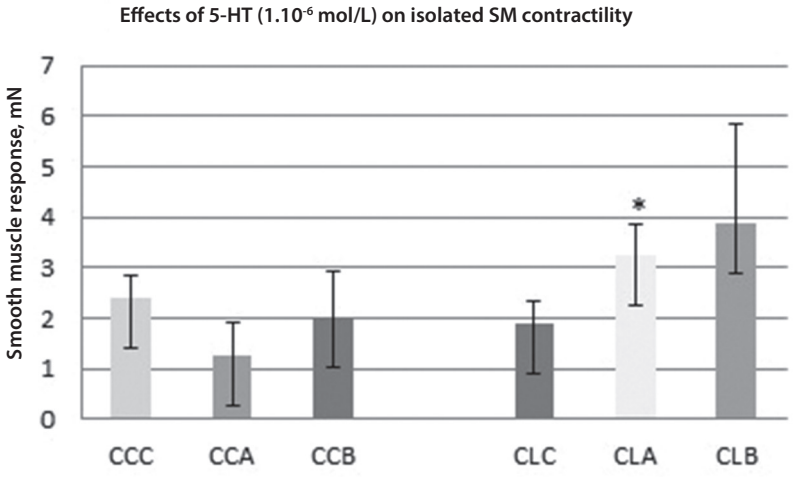

Figure 2. Contractile responses of isolated SM preparations to exogenously administered 5-HT (1.10^{-6} mol/L). CCC: circular column – control; CCA: circular column incubated with TCdA; CCB: circular column incubated with TCdB; CLC: longitudinal colon – control; CLA: longitudinal colon incubated with TCdA; CLB: longitudinal colon incubated with TCdB. Significant differences in reactions compared to the control were indicated by * ($p<0.05$).

a contraction of 3.24±0.61 mN, significantly increased compared to the control ($p<0.05$) without changes in the amplitude of phase reductions **(Fig. 2)**.

The SM preparations (n=10) incubated with 1.10^{-8} mol/L TCdB responded to exogenously administered 5-HT with a contraction of 3.88±1.97 mN without changes in the amplitude of phase reductions **(Fig. 2)**.

Effects of dopamine (1.10^{-6} mol/L) on contractile activity of isolated smooth muscle preparations from colon circulum and colon longitudinalis.

Colon circulum

In control preparations, administration of dopamine elicited a relaxation response of −2.41±0.43 mN (n=20) without significant changes in the amplitude of phase contractions **(Fig. 3)**.

The SM preparations (n=18) incubated with 1.10^{-8} mol/L TCdA responded to exogenously administered dopamine with a relaxation of −1.25±0.64 mN ($p>0.05$) without changes in the amplitude of phase contractions **(Fig. 3)**.

The SM preparations (n=10) incubated with 1.10^{-8} mol/L TCdB responded to exogenously administered dopamine with a relaxation of −3.88±1.97 mN without changes in the amplitude of phase contractions **(Fig. 3)**.

Colon longitudinalis

In control preparations, administration of dopamine elicited a relaxation response of −0.87±0.1 mN (n=20) without significant changes in the amplitude of phase contractions **(Fig. 3)**.

The SM preparations (n=15) incubated with 1.10^{-8} mol/L TCdA responded to exogenously administered dopamine with a relaxation of −0.35±0.45 mN without changes in the amplitude of phase contractions **(Fig. 3)**.

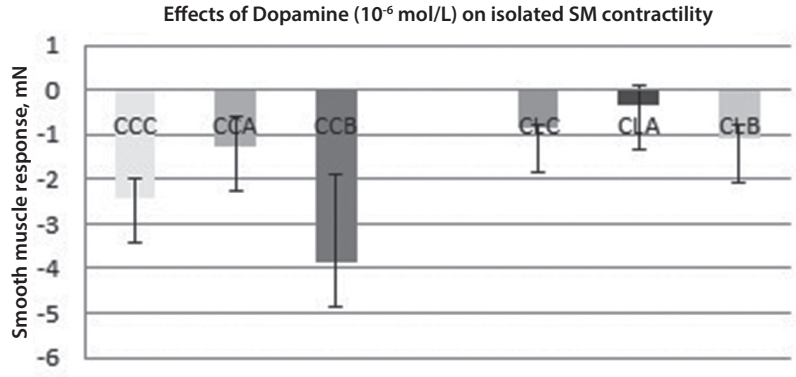

Figure 3. Reduction responses of isolated SM preparations to exogenously administered dopamine (1.10^{-6} mol/L). CCC: circular column – control; CCA: circular column incubated with TCdA; CCB: circular column incubated with TCdB; CLC: longitudinal colon – control; CLA: longitudinal colon incubated with TCdA; CLB: longitudinal colon incubated with TCdB. Significant differences in reactions compared to the control were indicated by * ($p<0.05$).

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The SM preparations (n=10) incubated with 1.10^{-8} mol/L TCdB responded to exogenously administered dopamine with a relaxation of −1.09±0.3 mN without changes in the amplitude of phase contractions **(Fig. 3)**.

Effects of norepinephrine (1.10^{-6} mol/L) on contractile activity of isolated smooth muscle preparations from the colon circulum and colon longitudinalis

Colon circulum

In control preparations, administration of norepinephrine elicited a relaxation response of −0.68±0.03 mN (n=20) without significant changes in the amplitude of phase contractions **(Fig. 4)**.

The SM preparations (n=18) incubated with 1.10^{-8} mol/L TCdA responded to exogenously administered norepinephrine with a relaxation of −0.6±1.01 mN without changes in the amplitude of phase contractions **(Fig. 4)**.

The SM preparations (n=10) incubated with 1.10^{-8} mol/L TCdB responded to exogenously administered norepinephrine with a relaxation of −0.88±0.9 mN without changes in the amplitude of phase contractions **(Fig. 4)**.

Colon longitudinalis

In control preparations, administration of norepinephrine elicited a relaxation response of −0.96±0.05 mN (n=20) without significant changes in the amplitude of phase contractions **(Fig. 4)**.

The SM preparations (n=15) incubated with 1.10^{-8} mol/L TCdA responded to exogenously administered norepinephrine with a relaxation of −0.63±0.14 mN without changes in the amplitude of phase contractions **(Fig. 4)**.

The SM preparations (n=10) incubated with 1.10^{-8} mol/L TCdB did not respond to exogenously administered norepinephrine **(Fig. 4)**.

DISCUSSION

Because the effects of Clostridium difficile toxins on smooth muscle contractility are a component of their overall impact on clinical phenomena, the possible mechanisms of affecting the gastrointestinal tract are of critical importance. The effects on intestinal muscle involved in the formation of segments along the small intestine (assuming that the contractility of the longitudinal muscles is not affected) should cause a change in the frequency and amplitude of segmentation. The above would lead to some disruption of the synchrony of movement of intestinal contents. This would inevitably cause atypical retention of intestinal contents in some areas and premature evacuation of others, combined with difficulty in homogenizing the masses in the intestinal lumen.[6]

The obtained results showed significant differences in the magnitude of the contractile responses to ACh (main parasympathetic regulator and major testing agent for muscle contractile vitality) in SM preparations incubated with TCdA compared to the control ones, while no differences were found in the reactions of the preparations treated with TCdB compared to the control ones. The contractile effects caused by ACh in the SM of gastrointestinal tract are mainly the result of activation of the M_3-cholinergic receptors.[7] Thus, the increase in SM tone is due to the release of Ca^{2+} from intracellular depots.[8,9] The observed increase in contractile response in colon preparations incubated with TCdA could be a consequence of the SM membrane depolarization observed by Gilbert upon action with TcdA.[10] In the causal aspect, this is related to the enhancement of Ca^{2+} influx, provoked indirectly by exogenously applied ACh. An argument in favor of our hypothesis is the reported increase in carbachol-induced contractions in SM treated with TCdA.[10] The above data suggest the presence of prerequisites for enhanced sensitivity of the muscles of the gastrointestinal tract to the regulatory function of ACh.

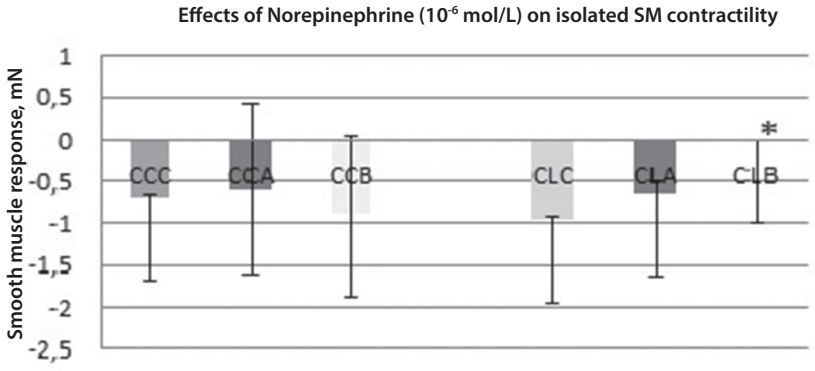

Figure 4. Reduction responses of isolated SM preparations to exogenously administered norepinephrine (1.10^{-6} mol/L). CCC: circular column – control; CCA: circular column incubated with TCdA; CCB: circular column incubated with TCdB; CLC: longitudinal colon – control; CLA: longitudinal colon incubated with TCdA; CLB: longitudinal colon incubated with TCdB. Significant differences in reactions compared to the control were indicated by * ($p<0.05$).

The lack of significant changes in ACh-induced responses in TCdB-incubated preparations should be analyzed on the background of data existing[11], namely: significant likelihood of TcdB inactivating Rho proteins involved in modulation of the contractile apparatus; regulating the activity of phospholipase D, with a negligible effect on the activity of protein kinase C as well as pronounced inhibitory effect on muscarinic receptor activity.[12]

The tests to determine the effect of TCdA and TCdB on 5-HT mediation were performed according to the same scheme as in the experiments with ACh. It was established that circular SM colon preparations demonstrated resistance to 5-HT-induced reactions in the presence of TCdA and TCdB. Rise of contractile reaction in the presence of TCdA was observed in longitudinal preparations.

Enteroendocrine cells are known to act as pressure sensors secreting 5-HT that initiates peristaltic reflexes.[13] The contraction-relaxation processes provoked by 5-HT through interneuronal interactions involve acetylcholine, substance P, nitric oxide (NO), vasoactive intestinal peptide (VIP), and calcitonin gene-regulating peptide.[14] The activation of Rho-protein and Rho-kinase is most probably a leading factor in the generation of 5-HT-induced contractions.[15] Meanwhile, a major regulatory link in the contractility chain – protein kinase C, plays a minor role in the development of these processes.[15] Our observations in this aspect contradict Lucius' hypothesis about the negative effect of 5-HT on the contractile activity of the longitudinal intestinal muscles in experimental animals.[16]

Gastrointestinal expression and function of 5-HT receptors are specific on different levels of the gastrointestinal tract in rats. 5-HT_7-receptor activation causes relaxation of SM probably through activation of cyclic nucleotides, whereas 5-HT_{2B}-receptors mediate the contractions. The other expressed type – 5-HT_4-receptor is likely to participate in both inhibition and activation of SM. Parallel neural and myogenic processes are involved in above mentioned effects.[17]

It remains unclear why the contractile reactivity of the longitudinal muscles in the area of the proximal colon is affected by TCdA intoxication while the contractile reactivity of the circular ones is not. It is likely that there are differences in the interneuronal interactions of serotonergic mediation leading to the release of ACh, substance P, NO, and VIP in the submucosal, and myenteric intramural plexuses.[3]

In contrast to the contractile character of effects caused by ACh and 5-HT, exogenously administered dopamine relaxes the SM of the colon. The magnitude of the effect is different in circular and longitudinal preparations; it is significantly weaker reaction in the latter. Applied to isolated SM from rat colon, dopamine induces a relaxation, which at high concentrations (10^{-4} mol/L) is accompanied by a complete loss of spontaneous contractile activity.[10] The effect is not related to direct activation of dopamine receptors but is probably mediated by β-adrenoceptors.

According to other evidence of a noncholinergic neuronal origin of its relaxation effect, dopamine inhibits SM contractions in the rat colon by activating β_1-adrenoceptors on intramural plexuses and by β_2-receptors expressed on SM itself.[18] Analogous experiments have shown that the inhibitory action of dopamine involves inhibition of ACh release from enteric neurons mediated by D_1 and D_2-receptors, as well as influencing NO activity and purinergic mediation.[19] Probably the dominant role in these processes is played by activation of adenylate cyclase and consequent increase in cyclic adenosine monophosphate concentration.

The lack of influence of TCdA and TCdB on dopamine-induced relaxations in our experiments allows some speculations: on the one hand, pronounced "non-competitiveness" in the involvement of the adenylate cyclase mechanism of toxins and catecholamines, and on the other, inability to stop relaxing SM processes due to the wide range of receptor and interneuronal interactions that have been described, dopamine activates at the enteric plexus.

We do not rule out that preservation of the relaxation effect caused by dopamine is the result of membrane hyperpolarization[20] of SM cells of the colon (concomitant enhanced K^+ current), which in itself turns the effect independent of specific intracellular contractile mechanisms. It was commented above that activation of β-adrenoceptors is one of the pathways leading to suppression of SM activity in the colon wall. The effect is due to increased synthesis of cyclic adenosine monophosphate, activation of protein kinase A, and blocking the ability of myosin light-chain kinase to initiate contractions.

As a neuronal regulator of enteric contractile activity, the sympathetic part of the autonomic nervous system interacts in a complex pattern, both with the parasympathetic part and with the structures of the nonadrenergic and noncholinergic innervating systems. An important element of that interaction at the rat colon level is the antagonism of noradrenaline and VIP activity.[21] In addition, noradrenaline and adrenaline have been shown to significantly reduce the effectiveness of ACh in the longitudinal intestinal layer excitation. The effect is receptor-mediated (α-adrenoceptors), and it does not involve dopamine, the other catecholamine that is important for the motility.[22]

As commented above, conditions such as colitis, dysbacteremia, gastrointestinal intoxications (including *Clostridium difficile* intoxication), and infections affect in a similar way certain units and agents of the SM contractile system of the tract, such as VIP and ACh. A study using a model of chemically-induced colitis[23] demonstrated a significant increase in acetylcholinesterase and VIP levels in the SM wall of the colon in rats.

Our experimental data related to the effect of exogenous norepinephrine on isolated SMs show a weak relaxation effect in both circular and longitudinal preparations. Preliminary treatment with TcdA and TcdB did not result in changes in responses, except in the case of a longitudinal colon preparation treated with TcdB in which no reaction at all was present.

An acceptable explanation for this is the above-mentioned complex intraplex interactions of sympathetic innervation, as well as the fact that the presence of norepinephrine

is associated with a decrease in VIP efficacy, while when the intestinal function is impaired, the peptide levels are significantly elevated. Such antagonism of influences can serve to extrapolate the direction of development of contraction and relaxation processes. In purely quantitative terms, it should be noted that exogenously administered norepinephrine is about four times less active than adrenaline.[22]

CONCLUSIONS

The developed experimental model provides data suggesting that TCdA and TCdB affect directly the contractile reactivity of isolated rat colon smooth muscle. TCdA exerts a stronger direct effect on smooth muscle sensitivity to acetylcholine and 5-HT than TCdB does. Such a trend has not been established for dopamine and norepinephrine.

For better understanding of the above and in order to clear some cellular mechanisms of the effects obtained, future experiments will include investigations with Real Time Cell Analyzer, which provides direct measurement of cell culture electric impedance, as well as measure of reaction of isolated smooth muscles to electrostimulation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. Gilbert RJ, Triadafilopoulos G, Pothoulakis C, et al. Effect of purified Clostridium difficile toxins on intestinal smooth muscle. Am J Physiol 1989; 256(4,Pt 1):759–66.
2. Wilcox M. Gastrointestinal disorders and the critically ill. Clostridium difficile infection and pseudomembranous colitis. Best Pract Res Clin Gastroenterol 2003; 17(3):475–83.
3. Hansen MB. The enteric nervous system II: gastrointestinal functions. Pharmacol Toxicol 2003; 92(6):249–57.
4. Browning JG, Hardcastle J, Hardcastle PT, et al. The role of acetylcholine in the regulation of ion transport by rat colon mucosa. J Physiol 1977; 272(3):737–54.
5. Gaginella TS, Grisham MB, Thomas DB, et al. Oxidant-evoked release of acetylcholine from enteric neurons of the rat colon. J Pharmacol Exp Ther 1992; 263(3):1068–73.
6. Fordtran JS, Rector FC, Locklear TW, et al. Water and solute movement in the small intestine of patients with sprue. J Clin Invest 1967; 46(3):287–98.
7. Ehlert FJ, Sawyer GW, Esqueda EE. Contractile role of M2 and M3 muscarinic receptors in gastrointestinal smooth muscle. Life Sci 1999; 64(6-7):387–94.
8. Van Koppen CJ, Kaiser B. Regulation of muscarinic acetylcholine receptor signaling. Pharmacol Ther 2003; 98(2):197–220.
9. Lucas JL, Wang D, Sadee W. Calmodulin binding to peptides derived from the i3 loop of muscarinic receptors. Pharm Res 2006; 23(4):647–53.
10. Aguilar HN, Mitchell HF. Physiological pathways and molecular mechanisms regulating uterine contractility. Hum Reprod Update 2010; 16(6):725–44.
11. Schmidt M, Rümenapp U, Bienek Ch, et al. Inhibition of receptor signaling to phospholipase D by Clostridium difficile toxin B: Role of Rho proteins. J Biol Chem 1996; 271(5):2422–6.
12. Schmidt M, Hüwe SM, Fasselt B, et al. Mechanisms of phospholipase D stimulation by M3 muscarinic acetylcholine receptors: evidence for involvement of tyrosine phosphorylation. Eur J Biochem 1994; 225(2):667–75.
13. Gershon MD. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. Curr Opin Endocrinol Diabetes Obes 2013; 20(1):14–21.
14. Hansen MB. The role of serotonin in intestinal luminal sensing and secretion. Acta Physiol (Oxf) 2008; 193(4):311–23.
15. Nishikawa Y, Doi M, Koji T, et al. The role of Rho and Rho-dependent kinase in serotonin-induced contraction observed in bovine middle cerebral artery. Tohoku J Exp Med 2003; 201(4):239–49.
16. Lucius C, Arner A, Steusloff A, et al. Clostridium difficile toxin B inhibits carbachol-induced force and myosin light chain phosphorylation in guinea-pig smooth muscle: role of Rho proteins. J Physiol 1998; 506:83–93.
17. Wouters MM, Farrugia G, Schemann M. 5-HT receptors on interstitial cells of Cajal, smooth muscle and enteric nerves. Neurogastroentero Motil 2007; 19:5–12.
18. Ek BA, Bjellin LA, Lundgren BT. Beta-adrenergic control of motility in the rat colon. I. Evidence for functional separation of the beta 1- and beta 2-adrenoceptor-mediated inhibition of colon activity. Gastroenterology 1986; 90(2):400–7.
19. Auteri M, Zizzo MG, Amato A, et al. Dopamine induces inhibitory effects on the circular muscle contractility of mouse distal colon via D1- and D2-like receptors. J Physiol Biochem 2016; 73(3):395–404.
20. Al-Jahmany AA, Schultheiss G, Diener M. Effects of dopamine on ion transport across the rat distal colon. Pflugers Arch 2004; 448(6):605–12.
21. Bhaskar M, O'Dorisio T, Cataland S, et al. Angiotensin II and norepinephrine antagonize the secretory effect of VIP in rat ileum and colon. Peptides 1984; 5(2):291–4.
22. Paton WD, Vizi ES. The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. Br J Pharmacol 1969; 35(1):10–28.
23. Kishimoto S, Haruma K, Tari A, et al. Rebamipide, an antiulcer drug, prevents DSS-induced colitis formation in rats. Dig Dis Sci 2000; 45(8):1608–16.

Воздействие токсинов *Clostridium Difficile* на реактивность гладкой мускулатуры толстой кишки крыс

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Резюме

Цель: Настоящее исследование было проведено в попытке найти возможные прямые механизмы действия токсинов *Clostridium difficile* A и B (TCdA и TCdB) на сократительную способность изолированных гладких мышц кишечника крыс, поскольку пути сокращения, на которые воздействуют токсины и которые отвечают за моторику расстройства остаются невыясненными.

Материалы и методы: В экспериментах использовали взрослых крыс-самцов линии Вистар. Выделяли препараты продольных гладких мышц (ГМ) проксимального отдела толстой кишки и изометрически регистрировали их сократительную активность. Образцы помещали в ванны для тканей и экзогенно обрабатывали ацетилхолином (ACh), серотонином (5-HT), дофамином, норадреналином, TCdA и TCdB. Была исследована способность TCdA и TCdB влиять на действие этих медиаторов на активность ГМ.

Результаты: Эксперименты показали, что возбуждающее действие АХ и 5-НТ на сократительную способность толстой кишки усиливается TCdA, а не TCdB. И наоборот, расслабляющий эффект дофамина и норадреналина на сократительную активность ГМ толстой кишки находится под влиянием TCdB, но не TCdA. TCdA оказывает более сильное прямое влияние на чувствительность ГМ in vitro к ACh и 5-НТ, чем TCdB.

Заключение: TCdA и TCdB непосредственно влияют на сократительную реактивность изолированных гладких мышц толстой кишки крыс. TCdA оказывает более сильное прямое влияние на чувствительность гладких мышц к ацетилхолину и 5-НТ, чем TCdB. Для дофамина и норадреналина такой тенденции не установлено.

Ключевые слова

Clostridium difficile, крыса, гладкая мускулатура