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Research Article

The role of tachykinin receptors in non-adrenergic non-cholinergic contractile responses of ileums of mouse, rat and guinea pig

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ABSTRACT

In our study, we tried to determine the role of tachykinins (TKs) in the non-adrenergic non-cholinergic (NANC) nervous system responses obtained with electrical field stimulation (EFS) in isolated rat, guinea pig and mouse ileum. The Neurokinin1 (NK1) receptor antagonist, RP 67580, the Neurokinin 2 (NK2) receptor antagonist, GR 159897, and the Neurokinin 3 (NK3) receptor antagonist, SB 222200, were applied in the concentration range of 10^{-8}-10^{-6}M. RP 67580 partially decreased the contraction components of the NANC nervous system responses in the isolated rat, guinea pig and mouse ileums in a dose-dependent manner. While GR 159897 partially decreased the contraction components of the NANC nervous system responses in the isolated rat ileums in a dose-dependent manner, it was ineffective in isolated guinea-pig ileum. SB 222200 minimally reduced the contraction components of the NANC nervous system responses in the isolated rat ileum at 10^{-6}M. It was ineffective on the contraction components of the isolated guinea pig and mouse ileums. As a conclusion, in our study while NK1 receptors are effective in 3 animal species, NK2 receptors are only effective in rats and NK3 receptors are ineffective in all 3 animal species for the contraction components of the NANC responses which are obtained with EFS in isolated rat, guinea-pig and mouse ileums. The possible reasons for the TK antagonists to have different effects in different animal species may be the difference between animal species, TK receptors' being localized in different places of ileum, species-dependent differences in receptor affinity between antagonists and different antagonists' being effective on different sub-types of NK1 and NK2 receptors.

Keywords: Isolated ileum; Longitudinal muscle; NK1 receptors; NK2 receptors; NK3 receptors; Non-adrenergic non-cholinergic nervous system.

INTRODUCTION

Tachykinins (TKs) are structurally peptide related neurotransmitter/neuromodulator substances that exist in respiratory, genitourinary, vascular systems and gastrointestinal (GI) tract of different animal species; and they show their effects by mediating many biological activities with their specific receptors in central nervous system and peripheral organs. The endogenous agonists of TKs are substance P (SP), Neurokinin A (NKA) and Neurokinin B (NKB). Their effects are regulated by 3 different receptors; NK1, NK2 and NK3, respectively; but it varies in different animal species (Croci et al., 1995). It is interesting that most of the information about TK systems is obtained from pathological cases. Most of the pre-clinical indicators showed that the TK receptors activate and/or maintain the indications and symptoms of diseases like Inflammatory Bowel Disease and Irritable Bowel Syndrome. No commercial medicine has been produced in the clinical experiments testing different TK receptors in human GI system until now. On the other hand, it creates an expectation that the TK receptor-based medicines will generate appropriate therapeutic effects in humans as well due to this medicines' competence in decreasing or preventing the receptor-mediated effects of TK in bowel diseases in the experimental animal models, with the condition that the potential adverse effects will be observed with maximum attention (Vannucchi and Evangelista, 2013 for a reviews).

TKs, contribute to the control of non-adrenergic non-cholinergic (NANC) nerves in GI system via their receptors. NK1, NK2, and NK3 receptors mediate isotonic contractions of TKs in both longitudinal and circular muscle layers of guinea pig ileum. It is known that TK NK1 receptors mediate non-cholinergic contractions of ileum's longitudinal muscle. However, it was reported that they also participate in the cholinergic muscular activity. TK NK3 receptors do not only contribute to the cholinergic contractions of ileum's longitudinal muscle, but they also contribute to its non-cholinergic contractions. On the other hand, TK NK1 and NK2 receptors mediate the non-cholinergic tonic and phasic contractions of ileum's circular muscle; and TK NK3 receptors mediate cholinergic muscular responses. It was

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also reported that TKs mediate phasic and muscular activity in the longitudinal muscle of rat ileum under isometric conditions (Vilain et al., 1997).

It was asserted that SP was the main contracting transmitter in the controlling of GI motility. Pharmacological studies in the isolated ileum showed that SP has powerful spasmogenic effects. SP implements these effects in smooth muscle membrane either directly, by interacting with both NK1 and NK2 receptors or indirectly, by neurogenic atropine-sensitive pathways (probably by interacting with NK3 receptors). On the other hand, it was reported in some studies that the NANC inhibitory transmitter release which is assumed to be stimulated by SP, or the inhibition of acetylcholine release causes relaxation of GI smooth muscle (Garcia-Villar et al., 1996).

In this study, we aimed to determine the role of TKs in the NANC responses obtained with EFS in isolated rat, guinea pig and mouse ileum. We also tried to determine whether the responses changed depending on the animal species and which type of TK receptors took part in the emerged responses.

MATERIALS AND METHODS

Animals

The ethical approval was taken from Trakya University, Faculty of Medicine, Ethical Committee before the experimental studies started. In total 60 guinea pigs (250-300 g), BALB/c mice (20-30 g) and Wistar albino rats (200-300 g) which were provided from Trakya University, Unit of Experimental Animals were used in the experiments. All of the animals were adult and male. Animals were raised under the conditions of 50-60% humidity rate, 22±1°C temperature for 12 hours night/12 hour's day. The animals, which were brought to the laboratory at least one week before the experiments, were kept in a noiseless environment with 22±1°C room temperature, without food restriction. The animals were not given any food one day before the experiments.

Drugs

Atropine (Sigma), Phentolamine (Sigma), Propranolol (Sigma), RP 67580 (Tocris), GR 159897 (Tocris), SB 222200 (Tocris), Tetrodotoxin (TTX) (Tocris), Ketalar (Eczacıbaşı) The NK1 receptor antagonist, RP 67580, the NK2 receptor antagonist, GR 159897, and the NK3 receptor antagonist, SB 222200 and TTX which is a neurotoxin were dissolved in ethanol.

Organ Bath

After the rat, guinea pig and the mouse were anesthetized with ketamine (60-120 mg/kg dose i.m.), the ileocecal sphincter was found, then 10 cm distal part of it was left and 10 cm ileum part was taken and put to a petri dish containing Krebs solution. Decapitation was used as the euthanasia method. After the tissues around the bowel were cleaned, four ileal segment pieces, each 1-2 cm long, were cut and tied from the lower and upper ends longitudinally with keeping the lumen open, were hung to the 30 ml organ baths that were aired with mixture of 95% O_2 + 5% CO_2 and contained Krebs solution in 37°C temperature. The composition of Krebs solution was as follows: NaCl 118 mmol/L, KCl 4.70mmol/L, $CaCl_2$ 2.52 mmol/L, $MgSO_4$ 1.64 mmol/L, $NaHCO_3$ 24.88 mmol/L, KH_2PO_4 1.18 mmol/L, glucose 5.55 mmol/L. It was applied 2 g of preload to the ileum segments of rats and 1 g of preload to the ileum segments of guinea pigs and mice. Responses were recorded using a polygraph (Grass Model 7 Polygraph) and isometric transducers (Grass FT03). EFS was applied using a computer controlled stimulator (MAY ST95PT Simulator) over the electrodes placed in parallel to ileum segments. In the study, EFS values of 20 Hz frequency, 0,8msec pulse width, 50 V were used for 20 seconds at 10-minute intervals. During the experiments 15 minutes before the application of the medicines, muscarinic receptor antagonist (atropine, 3 μM), α-adrenergic receptor blocker (phentolamine, 5 μM), and β-adrenergic receptor blocker (propranolol, 5 μM) were applied into the organ bath to prevent emerge of cholinergic and adrenergic responses. In our study, 3 different antagonists were used in 3 different animal species; GR 159897 (n=8), RP 67580 (n=8), and SB 222200 (n=8).

Statistical Analysis

Graph pad Prism 6 for Mac program was used for the analysis of the data. Concentration-response graphics were obtained and two way repeated measures Anova followed by the post-hoc Bonferronni was applied. In the statistical analyses, $p<0.05$ values were presumed as significant.

RESULTS

TK NK1 receptor antagonist, RP 67580 partially decreased the contraction components of the NANC responses in the isolated rat, guinea-pig and mouse ileums in a dose-dependent manner (Figure 1); there was no statistically significant difference between responses.

TK NK2 receptor antagonist, GR 159897 partially decreased the contraction components of the NANC responses in the isolated rat ileums in a dose-dependent manner. The dose-dependent effect of GR 159897 in the contraction components of the NANC responses in isolated mouse ileums was lesser when compared to rat; but it was slightly higher than those of guinea pig. GR 159897 was ineffective on the contraction components of the NANC responses in isolated guinea pig ileum (Figure 2). According to rat, there was a statistically significant difference between responses of guinea-pig ($p<0.0001$) and mouse ($p<0.0001$) at 1.10^{-8} M. There was no statistically significant difference between guinea-pig and mouse at 1.10^{-8} M. According to rat, there was a statistically significant difference between responses of guinea-pig ($p<0.0001$) and

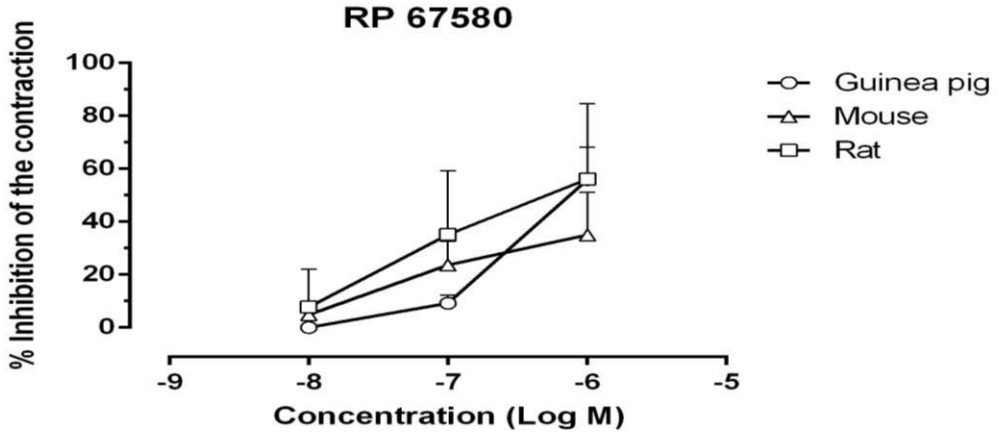

Figure 1: The effect of the NK1 receptor antagonist RP 67580 in contraction components of the isolated rat, guinea pig and mouse ileums(n=8, vertical bars show standard error of the mean).

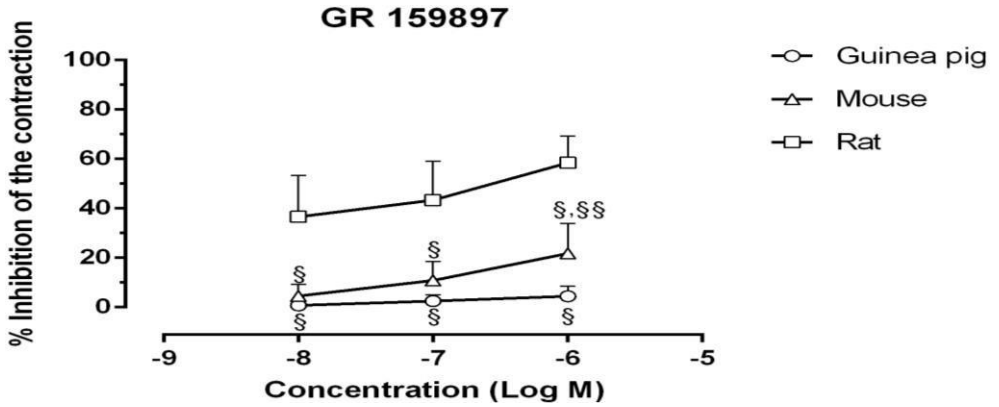

Figure 2: The effect of the NK2 receptor antagonist, GR 159897 in contraction components of the isolated rat, guinea pig and mouse ileums.§:p<0.0001against rat;

§§: p<0.0001 against guinea-pig; (Repeated measures, two-way ANOVA, post-hoc Bonferroni test) (n=8, vertical bars show standard error of the mean).

mouse (p<0.0001) at 1.10^{-7}M. There was no statistically significant difference between guinea-pig and mouse at 1.10^{-7} M. According to rat, there was a statistically significant difference between responses of guinea-pig (p<0.0001) and mouse (p<0.0001) at 1.10^{-6} M. There was statistically significant difference between-guinea-pig and mouse at 1.10^{-6}(p<0.05).

TK NK3 receptor antagonist, SB 222200 minimally reduced the contraction components of the NANC responses in the isolated rat ileum at 10^{-6}M. But it was ineffective on the contraction components of NANC responses at 10^{-7} and 10^{-8} M. It was also ineffective on the contraction components of NANC responses of the isolated guinea pig and mouse ileums (Figure 3). According to rat, there was a statistically significant difference between responses of guinea-pig (p<0.0001) and mouse (p<0.05) at 1.10^{-6}M.

Neuron blocker, TTX decreased the contraction components of the NANC responses in isolated rat ileums in a dose-dependent manner. It completely abolished the contraction components of the NANC responses at 10^{-6}M. TTX dose-dependently decreased the relaxation components of the NANC responses in the isolated rat ileum at the concentration range of 3.10^{-8}M to 10^{-6}M (Figure 4). It also dose-dependently decreased the contraction and relaxation components of the NANC responses in isolated mouse ileums, completely abolishing the responses at 10^{-6}M (Figure 5). TTX dose-dependently decreased the contraction components of the NANC responses in isolated guinea pig ileums (Figure 6). In contrast to responses obtained in ileums of rats and mice, EFS did not produce a relaxation in ileal segments of guinea pigs.

DISCUSSION

Electrical stimulus may induce contraction or relaxation in longitudinal and circular smooth muscle layer. And this suggests that ileum NANC transmission has both inhibitory and excitatory effect. The role of different transmitters in the relaxation or contraction of NANC should be discussed (Bauer and Matusak, 1986, Gustafson et al., 1990, Knudsen and Tottrup, 1991, Osthaus and Galligan, 1992, Radomirov and Venkova, 1988, Williams and Parsons, 1995).

The blockage of the NANC EFS-induced responses by TTX shows that these responses are neuronal origin. It might be said that there is a mutual interaction be-

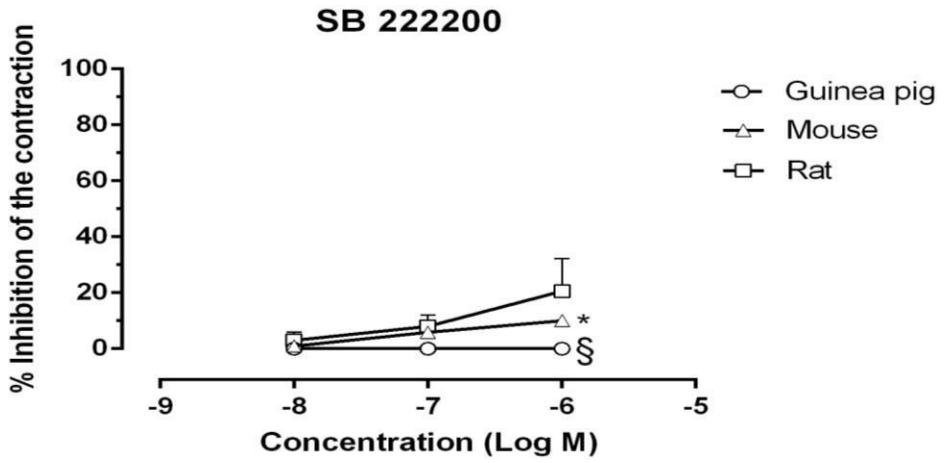

Figure 3: The effect of the NK3 receptor antagonist, SB 222200 in contraction components of the isolated rat, guinea pig and mouse ileums.

*: p<0.05 against rat; §:p<0.0001against rat; (Repeated measures, two-way ANOVA, post-hoc Bonferroni test) (n=8, vertical bars show standard error of the mean).

tween NANC neurotransmitters and NANC neuron mechanisms in the pattern of NANC components (Ivancheva et al., 1997). The twitch responses caused by EFS in myenteric plexus-smooth muscle preparations of guinea pig ileum were abolished by TTX (1µM) (Croci et al., 1995). In our study, TTX completely abolished the contraction and relaxation components of NANC responses in the isolated rat and mouse ileums at 10^{-6}M. TTX prominently decreased the contraction components of the NANC responses in isolated guinea pig ileums in a dose-dependent manner.

SP (Ivancheva et al., 1997, Costa M et al., 1985) and Adenosine triphosphate (Ivancheva et al., 2000) were considered responsible for the tonic contraction component of the NANC response which was obtained by 20 sec application of EFS after the blockage of cholinergic and adrenergic systems and nitric oxide (Ivancheva et al., 1997, 2000) was considered responsible for the relaxation component. In a study conducted by Maggi et. al., NK1 receptor selective antagonist, SR 140333 (0.1 µM, 60 minutes), caused partial inhibition in the contraction responses of isolated rat ileum circular muscle preparations induced by EFS. NK1 receptor selective antagonist, SR 140333 and NK2 receptor selective antagonist, MEN 10,627 together nearly abolished NANC contractions caused by EFS in ileum. According to the results of the study conducted by Maggi et. al., the major role of endogenous TKs is to act like an excitatory NANC transmitter in the circular muscle of rat small bowel. In addition, the effects of powerful selective receptor antagonists SR 140333 and MEN 10,627 support the result that both NK1 and NK2 receptors are functionally activated by endogenous TKs in NANC contractions caused by EFS. In our study, NK1 receptor antagonist, RP 67580 partially decreased the contraction components of the NANC responses caused by EFS in the isolated rat ileum (56.2%). The common finding of our study and the study conducted by Maggi et al is that NK1 receptor antagonists partially decreased the contraction components of the NANC responses caused by EFS in the isolated rat ileum. However, the study conducted by Maggi et. al. is on circular muscle contraction. Since the technique we used records the contractions of longitudinal muscle, this condition should be taken into consideration while interpreting the results. In a study conducted by Maggi et. al., NK2 receptor selective antagonist, MEN 10,627 (0.1 µM, 60 minutes) caused partial inhibition in the contraction responses of circular muscle preparations of isolated rat ileum induced by EFS (MaggiandGiuliani, 1995). In our study, NK2 receptor antagonist, GR 159897 partially decreased the contraction components of the NANC responses caused by EFS in longitudinal muscle preparations of the isolated rat ileum (58.5%). TK NK3 receptors of rat are quite resistant to specific antagonists (Croci et al., 1995). In our study we detected that, NK3 receptor antagonist, SB 222200, minimally decreased (20.6%) the contraction components of the NANC responses in longitudinal muscle preparations of the isolated rat ileum at 10^{-6}M.

In a study of Ivancheva et. al., a NK1 receptor antagonist, AP 13.2 ACOH, decreased both twitching and tonic contraction of the the NANC contraction components in longitudinal smooth muscle of isolated guinea pig ileum. There are multiple inhibitory and excitatory mechanisms in the guinea pig ileum. Inhibitory purinergic and nitrergic mechanisms regulate the relaxation and contraction and they cause mutual interaction between NANC inhibitory and excitatory motor pathways (Ivancheva et al., 2000). In our study, NK1 receptor antagonist, RP 67580, partially decreased the contraction components of the NANC responses caused by EFS in the isolated guinea pig ileum (56%). Our study supports the finding reported by Ivancheva et al that

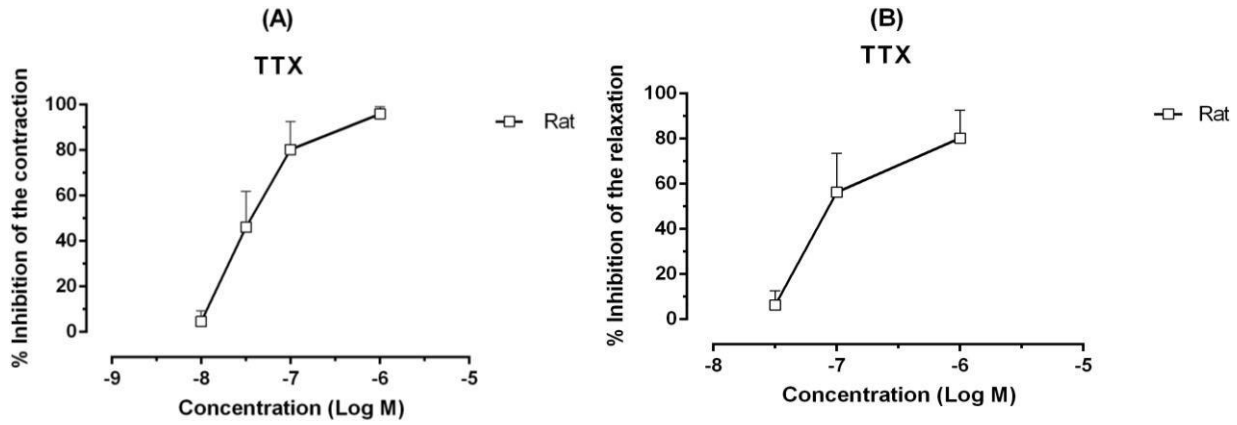

Figure 4: The effect of TTX in contraction (A) and relaxation (B) components of the isolated rat ileums (n=8, vertical bars show standard error of the mean).

NK1 receptor antagonists decrease the contraction components of the NANC responses. In our study we found that NK2 receptor antagonist GR 159897 was ineffective in the contraction components of the NANC responses caused by EFS in longitudinal muscle preparations of the isolated guinea pig ileum (it was observed that there was a decrease at the ratio of 4.5%). The studies in the bowel of guinea pig localized the immunore activity of NK3 receptor to the intrinsic primary sensory neurons (Mann et al., 1999, Jenkinson et al., 1999, Johnson et al., 1998). It was reported that NK3 receptor antagonist SR 142801 also has non-specific effects since it decreases basal electrical twitch responses in my enteric plexus longitudinal muscle preparations of guinea pig ileum and prevents the contractions caused by nicotine in the same tissue at higher concentrations (0.3-3µM). The non-specific effects of NK3 receptor antagonists SR 142801 and SR 142806 may be connected to the relationship of the mixture with verapamil-sensitive calcium channels or opioid receptors (Costa et al., 1985). In our study, NK3 receptor antagonist SB 222200 was ineffective in the contraction components of the NANC responses caused by EFS in longitudinal muscle preparations of the isolated guinea pig ileum.

The results obtained from isolated mouse ileum show that while the response to the TKs applied as exogenous in the mouse ileum are organized by both NK1 and NK2 receptors, the responses stimulated by nerve stimulation in the presence of atropine and guanethidine are organized by primarily NK1 receptors and the primary TK receptor that participates in the excitatory NANC transmission in mouse is NK1 receptor (Saban et al., 1999). While Saban et al claimed that NK1 receptors were the effective primary TK receptor to excitatory NANC (Saban et al., 1999), Lecci et. al. predicted that both NK1 and NK2 receptors participate in excitatory NANC (Lecci et al., 1998). In the study of Saban et. al, since the mouse ileal segments were horizontally hung, the obtained contraction and relaxation responses are related to the circular muscle. In our study the responses taken are related to the longitudinal muscle and this condition should be taken into consideration while the results are compared. In a study conducted by De Schepper et al in circular muscle of isolated mouse ileum, it was determined that NK1 receptor antagonist, RP 67580, prominently (but not completely) inhibited the contraction of NANC responses caused by EFS and RP 67580 (1µM) prevented the contractions caused by selective NK1 receptor antagonist, septide (De Schepper et al., 2005). NK1 receptor antagonists MEN 11467 (1µM) and L 733060 (1µM), who were evaluated in previous experiments, prominently failed to block the contractions caused by the septide. Species-dependent differences in NK1 receptor affinity between antagonists may be responsible for this condition. Similar to the findings obtained from rat and guinea pig small bowel, it might be concluded that the TK NK1 and NK2 receptors are the primary receptors that respond to NANC excitatory mediators in mouse ileum. Also the existence of NK1 receptor is detected in nitrergic myenteric neurons at this section of mouse bowel (De Schepper et al., 2005).

When De Schepper et. al analyzed the effects of TK receptor antagonists in the contractions caused by EFS, they detected that NK1 receptors had important effects. Also the findings of De Schepper et. al. are related to the contraction and relaxation responses of circular muscle layer. Since the findings we obtained in our study are related to the longitudinal muscle, this condition should be taken into consideration when comparing our findings to mentioned findings. In fact, our findings related to longitudinal muscle can be approached as supplementary for the findings of other studies related to the circular muscle. In summary, we can say that NK1 receptors have an important role in the NANC system that organizes either circular or longitudinal muscle contraction responses in mouse ileum. In the study conducted by Saban et. al. NK2 receptor antagonist SR48968 prominently blocked the NKA responses

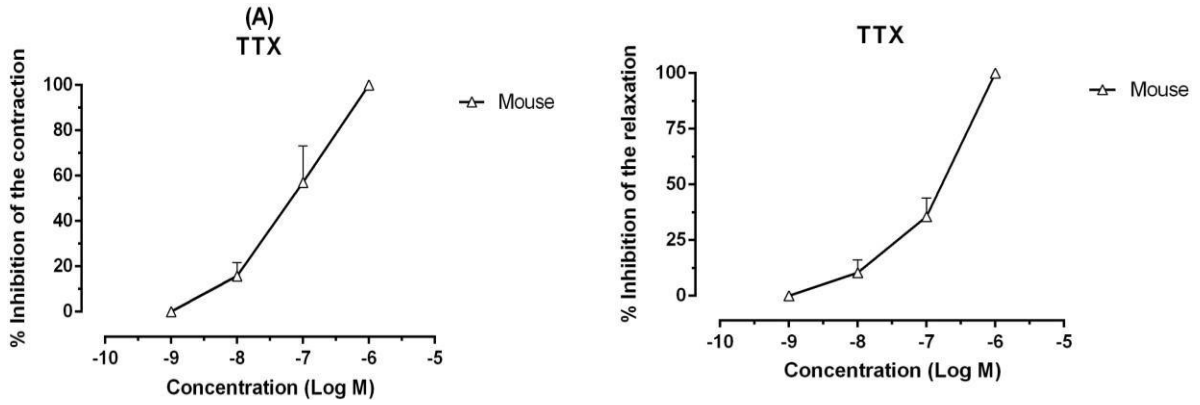

Figure 5: The effect of TTX in contraction (A) relaxation (B) components of the isolated mouse ileums (n=8, vertical bars show standard error of the mean).

in the circular muscle preparations of isolated rat ileum. In the study of De Schepper et. al., NK2 receptor antagonist nepadutant and NK3 receptor antagonist SR 142801 did not affect the NANC contractions by themselves but increased the inhibition of the NANC contractions in the circular muscle of isolated mouse ileum with RP 67580 which decreases the contraction of the NANC responses caused by EFS. All of the SP concentrations that were studied in the combined presence of nepadutant and SR 142801 were adequate in decreasing the contractions. However each antagonist did not have a prominent effect by itself. And it means that the blockage of neuronal and muscular NK1 receptors on the one hand or the blockage of muscular NK2 receptors on the other hand and concomitantly the inhibition of neuronal NK3 receptors cause an effective decrease in tachykinergic contraction. Contractions caused by SP are not affected by nepadutant (De Schepper et al., 2005). NK2 receptor antagonist, nepadutant (1μM) successfully decreases the contractions caused by β-A-NKA (1nM-100nM) (De Schepper et al., 2005). Nitric oxide synthase inhibition increases the contractions that were decreased by TTX addition. This supports the idea that there is an interaction between NK2 receptors and NANC nitrergic neurons. In our study, NK2 receptor antagonist GR 159897 minimally (21.8%) decreased the contraction components of NANC responses caused by EFS in the longitudinal muscle preparations of isolated mouse ileum at 10^{-6}M. In the study conducted by De Schepper et al it was detected that NK3 receptor antagonist SR 142801 did not affect NANC contractions by itself, but supported the effect of NK1 antagonist RP 67580 which decreased NANC contractions (De Schepper et al., 2005). Similarly in our study, NK3 receptor antagonist SB 222200 is found to be ineffective in the contraction components of the NANC responses caused by EFS in the longitudinal muscle of isolated mouse ileum.

There may be a difference among the affinities of TK antagonists depending on the animal species. For example, the affinity of MEN 10376 is more than the affinity of L-659877 in the preparations of rabbits, guinea pigs, cattle and humans. The opposite is observed in the preparations of hamsters and rats (Lecci, 2003 for a review). The cyclic peptide, MDL 29,913 (Burcher et al., 1991) has a higher affinity in rat and hamster preparations; and lower affinity in dog, rabbit, guinea pig and human (inactive at 2 μM) preparations (Lecci, 2003 for a review, Burcher et al., 1991, Mussap et al., 1996). At least 2 different sub-types of NK2 receptors in different mammal tissues have been defined as NK-2A and NK-2B in different species (Maggi et al., 1992). While NK-2A form is expressed in the smooth muscle of rabbit, guinea pig and human, NK-2B form is expressed in the smooth muscle of rat and hamster (Maggi et al., 1992). The NK2 antagonist we used in our study, GR 159897 may be effective in the NK-2B form of NK2 receptors. And this may be one of the possible reasons that NK2 receptor antagonist, GR 159897 was ineffective in the contraction components of the NANC responses in the isolated guinea pig ileum. In addition, the difference between the animal species can be explanatory for muscle type (circular, longitudinal) and organ type of differences (Saban et al., 1999).

The study conducted by Saban et al pointed out that the relaxation components of NANC caused by EFS is nitrergically organized in mouseileum just as in human and other species (Saban et al., 1999). No difference was detected between the wild type mouse and NK1-R knock out mouse regarding to TTX-sensitive relaxation responses. While the NK2 receptor antagonist, SR 48968 and NK3 receptor antagonist, SR 142801 were ineffective in wild mouse, NK1 receptor antagonist, CP-99994 increased the relaxation caused by EFS, and it made this by decreasing the contraction component of the respond which was presumably directed to EFS. Saban et. al. detected that the excitatory NANC response they obtained from the isolated mouse ileum was produced by NK1 receptors and that in the relaxation response, TKs did not have a part and this effect was nitrergic (Saban et al., 1999). In our study it was seen that NK1 receptor antagonist RP 67580, NK2 receptor antagonist GR 159897 and NK3 receptor antagonist SB 222200 did not have any prominent effects in

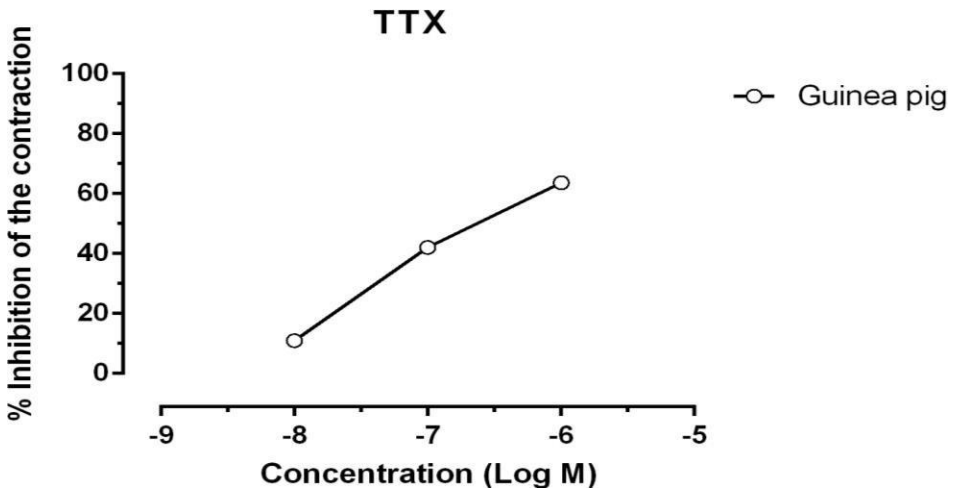

Figure 6: The effect of TTX in contraction components of the isolated guinea pig ileums (n=8, vertical bars show standard error of the mean).

the relaxation components of NANC responses in isolated rat, guinea pig and mouse ileums. It should be taken into consideration that the relaxation responses are related to ileum circular muscle in the mentioned studies and our responses are taken from longitudinal muscle.

The localization studies about TK receptors in guinea pig show that NK1 receptors localize mostly in myenteric/cholinergic nerves and interstitial cells of Cajal and slightly in the smooth muscle cells. On the other hand, NK2 receptors are mostly limited in smooth muscle cells. The immunohistochemical studies in rat and guinea pig intestine show that NK3 receptors localize especially in tachykinergic and cholinergic neurons, and in smooth muscle cells and interstitial cells of Cajal (Vannucchi and Faussone-Pellegrini 2000, Portbury et al., 1996, Portbury et al., 1996). In their functional studies in mouse ileum's circular muscle, De Schepper et al. detected the existence of NK1 receptors in nitrergicmyenteric neurons and smooth muscle cells in addition to cholinergic neurons (De Schepper et al., 2005), the existence of NK2 receptors in smooth muscle cells and nitrergic neurons and the existence of NK3 receptors in neurons (De Schepper et. al., 2005). However, they functionally failed to localize the NK3 receptors on smooth muscle surface (De Schepper et al., 2005). The data determined with immunohistochemistry were partially same but partially different from the functional observations of De Schepper et. al (De Schepper et al., 2005). Vannucchi et. al. detected that the distribution of NK1 and NK3 receptors were similar in rat, guinea pig and mouse (Vannucchi and Faussone-Pellegrini 2000); while NK1 receptors existed in interstitial cells, submucous and myenteric plexus neurons of Cajal, they were not observed in smooth muscle cells. (Vannucchi and Faussone-Pellegrini 2000) NK3 receptors were detected both in the smooth muscle cells of longitudinal and circular muscle layer and the neurons of each plexus. NK2 receptors were found in smooth muscle cells in all species. However, it was not found in nerve endings in mouse when compared to guinea pig and rat. An obvious difference between pharmacological and immunohistochemical data was the functional existence of NK1 receptors in ileal smooth muscle cells but this condition was not detected immunohistochemically. This may be linked to scantiness of receptors or the existence of different types of NK1 receptors that show a different ligand affinity (Vannucchi and Faussone-Pellegrini 2000).

CONCLUSIONS

To summarize, in our study while the NK1 receptors participate in the contraction components of the NANC responses induced by EFS in rat, mouse and guinea pig ileums, the NK2 receptors are only effective in rats and the NK3 receptors are ineffective in all three species. The possible reasons that TK antagonists show different effects in different animal species may be the usage of different EFS values in the studies, the difference between animal species, muscle (circular, longitudinal) or organ type, localization of TK receptors in different areas of ileum in animal species, species-dependent differences in receptor affinity between antagonists and the effectiveness of different antagonists on different sub-types of NK1 and NK2 receptors.

ACKNOWLEDGEMENTS

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