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Original
Role of muscarinic receptors in the contraction of jejunal smooth muscle in the horse: An in vitro study / Menozzi, Alessandro; Pozzoli, Cristina; Poli, Enzo; Bontempi, Giada; Serventi, Paolo; Meucci, Valentina; Intorre, Luigi; Bertini, Simone. - In: RESEARCH IN VETERINARY SCIENCE. - ISSN 0034-5288. - 115:(2017), pp. 387-392. [10.1016/j.rvsc.2017.07.012]

Availability:
This version is available at: 11381/2828782 since: 2021-10-06T11:00:12Z

Publisher:
Elsevier B.V.

Published
DOI:10.1016/j.rvsc.2017.07.012

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(Article begins on next page)

13 August 2025

Accepted Manuscript

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PII: S0034-5288(16)30707-X
DOI: doi: 10.1016/j.rvsc.2017.07.012
Reference: YRVSC 3380

To appear in: *Research in Veterinary Science*

Received date: 30 November 2016
Revised date: 9 June 2017
Accepted date: 8 July 2017

Please cite this article as: Alessandro Menozzi, Cristina Pozzoli, Enzo Poli, Giada Bontempi, Paolo Serventi, Valentina Meucci, Luigi Intorre, Simone Bertini , Role of muscarinic receptors in the contraction of jejunal smooth muscle in the horse: An in vitro study, *Research in Veterinary Science* (2017), doi: 10.1016/j.rvsc.2017.07.012

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Role of muscarinic receptors in the contraction of jejunal smooth muscle in the horse: an in vitro study

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Masked for review

Macello Malvisi (Noceto, Parma, Italy)

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ABSTRACT

Nonselective antimuscarinic drugs are clinically useful in several pathologic conditions of horses, but, blocking all muscarinic receptor (MR) subtypes, may cause several side effects. The availability of selective antimuscarinic drugs could improve therapeutic efficacy and safety.

We aimed to enlighten the role of different MR subtypes by evaluating the effects of nonselective, and selective M_1, M_2 and M_3 MR antagonists on the contractions of horse jejunum.

Segments of circular muscle of equine jejunum, were put into organ baths, connected to isotonic transducers, and the effects on ACh concentration-response curves, and on electrical field stimulation (EFS)-evoked contractions of intestinal preparations, induced by nonselective or selective MR antagonists, compared to pre-drug level, were studied. Atropine (nonselective MR antagonist), pirenzepine (selective M_1 antagonist), and p-FHHSiD (selective M_3 antagonist) competitively antagonized ACh (pA_2=9.78±0.21; 7.14±0.25 and 7.56±0.17, respectively). Methoctramine (selective M_2 antagonist) antagonized ACh in a concentration-unrelated fashion; however, it competitively antagonized carbachol, a nonselective muscarinic agonist (pA_2=6.42±0.23). Atropine dose-dependently reduced EFS-evoked contractions, reaching a maximal effect of -45.64±6.54%; the simultaneous block of neurokinin receptors, almost completely abolished the atropine-insensitive contractions. p-FHHSiD dose-dependently reduced EFS-induced contractions, while pirenzepine caused a minor decrease. Methoctramine, ineffective up to 10^{-7}M, enhanced the contractions at 10^{-6}M; the block of neurokinin receptors abolished the increase of contraction. Cholinergic contractions of horse jejunum are mainly mediated by M_3 receptors; M_2 selective antagonists seem to scarcely affect cholinergic, and to enhance neurokininergic contractions of equine jejunum, thus their use entails a lower risk of causing intestinal hypomotility, compared to nonselective drugs.

Keywords:

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Horse

Jejunum

Small intestine

Muscarinic receptor

Neurokinin receptor

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1. Introduction

Muscarinic receptor antagonists are used in horses for several different clinical purposes, such as to induce brochodilation in animals with recurrent airway obstruction (Hoffman et al., 1993), to treat painful bowel spasms related to colics (Plummer, 2009), or to prevent the bradycardia induced by the administration of selective α_2-agonists (Singh et al., 1997; Marques et al., 1998). Drugs such as atropine, glycopyrrolate or N-butylscopolammonium bromide are usually administered to horses in order to block the effects of acetylcholine (ACh) on muscarinic receptor (MR)s, but since these compounds are nonselective muscarinic antagonists, they may cause many side effects like tachycardia, blurred vision, urinary retention and gastrointestinal hypomotility (Greenblatt and Shader, 1973; Sundra et al., 2012). This latter adverse effect could also lead to serious conditions in the horse, such as paralytic ileus, especially following surgical procedures (Ducharme and Fubini, 1983). There is, to date, poor availability of selective muscarinic ligands which could be clinically useful, and even though nonselective muscarinic antagonists have been shown to be effective and relatively safe in most indications, the incidence of side effects is high, and therefore it is of great importance to expand the knowledge about the functional role of MR subtypes in different tissues and organs. Unfortunately, the lack of selective agonists for different MR subtypes is deeply complicating the matter, and the characterization of the five known MR subtypes (namely M_1, M_2, M_3, M_4 and M_5) has relied almost entirely on the differences in affinity of such receptors for muscarinic antagonists (Caulfield, 1993; Eglen, 2005). There is a great amount of collected evidence showing that, in smooth muscle cells of several species, including horse and man, both M_2 and M_3 subtypes are expressed, and that M_2 receptors are more abundant with respect to M_3 receptors in a 4:1 ratio (Ehlert et al., 1997). However, functional studies have discovered that the contraction of smooth muscle in gastrointestinal system and other organs, is instead mainly due to M_3 receptor activation (Barocelli et al., 1993; de Ponti et al., 1993; Eglen, 1996; Marti et al., 2005;

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Takeuchi et al., 2007; Teixeira-Neto et al., 2012; Menozzi et al., 2014), thus leading researchers to further investigate the reasons behind this apparent incongruity, and the real role of M_2 receptor subtype in the regulation of smooth muscle functions.

As for the signal transduction pathways, M_3 receptors are coupled with G_q proteins, and induce direct contraction of smooth muscle cell mainly by inositol triphosphate-mediated increase of intracellular calcium (Candell et al., 1990); M_2 receptor subtype inhibits adenylyl-cyclase activity via G_i proteins, and it opposes to the relaxation of contractile machinery induced by cyclic adenosin-monophosphate (cAMP) synthesized by sympathetic stimulation (Zhang and Buxton, 1991; Ehlert and Thomas, 1995). This framework is further complicated by the possible role of MRs located on neurons of myenteric plexus, which could modulate the effects of neurotransmitters, by either reducing or enhancing their release from nerve terminals. Indeed, pre-synaptic M_2 receptors on cholinergic neurons have been found in myenteric plexus of guinea pig and mouse, where they negatively regulate ACh release (North et al., 1985; Takeuchi et al., 2005).

This study was aimed to broaden the knowledge about the functional role of MR subtypes in horse small intestine, by evaluating the effects of nonselective and selective MR antagonists on ACh- or electrically-induced contractions of isolated samples of jejunum. The better understanding of functional role of different MR subtypes would be essential for the development of selective antimuscarinic drugs with better efficacy and safety profiles.

2. Materials and methods

2.1 Drugs

Tetrodotoxin (TTX) (neuronal sodium channel blocker), ω-conotoxin (CTX) GVIA (neuronal calcium channel blocker), ACh, atropine sulfate (nonselective muscarinic antagonist),

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pirenzepine (selective M_1 antagonist), methoctramine (selective M_2 antagonist), p-fluoro-hexahydro-sila-diphenidol hydrochloride (p-FHHSiD) (selective M_3 antagonist), and carbachol (nonselective muscarinic agonist) were purchased from Sigma Aldrich (Sigma Chemical Co., St Louis, MO, USA). Neurokinin receptor antagonists, L-732,138 (selective NK_1 antagonist), GR159897 (selective NK_2 antagonist), and SB218795 (selective NK_3 antagonist) were purchased from Tocris (Tocris Bioscience, Minneapolis, MN, USA).

All compounds were dissolved and diluted to the final concentration in distilled water, except neurokinin antagonists, which were first dissolved in 1% dimethyl sulfoxide (DMSO) and then diluted to the final concentration with distilled water. Such concentration of DMSO was previously tested and did not modify the contractility of preparations or the effect of drugs. All solutions were freshly prepared before each experiment and aliquots (10 to 100 μL) were added to the organ baths to achieve the desired molarity.

2.2 Preparations of tissues and intestinal motility in vitro

Tissues were collected from 48 healthy male horses (2-7 years of age), slaughtered at a private abattoir, 'masked for review'; the experiments were performed between September 2015 and August 2016. Segments of jejunum (10 cm long) were excised and rinsed with cooled (4°C) modified Krebs-Henseleit Solutions (KHS) of the following composition (mM): NaCl 113.0, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl2 \cdot 2H_2O$ 1.8, KH_2PO_4 1.2, $NaHCO_3$ 25 and dextrose 11.2.

The samples were stored in cooled KHS for the 10 min-transport from the slaughterhouse to the laboratory, where the intestinal specimens were carefully rinsed with fresh KHS and cleaned from the surrounding tissue and mucosa. Each segment of small intestine was subsequently cut in circularly-oriented strips (20 × 2 mm), tied to both ends with silk threads and then set up into 10 mL organ baths at 37 °C, containing the solution above described, gassed with 95% O_2 and 5% CO_2

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(pH 7.4). After a period of stabilization (60-120 min), the mechanical activity was measured by means of an isotonic transducer connected to the preparation, developing a passive stretch of 2-3 g throughout the entire experiment, and measured in cm of length modification of intestinal sample.

The preparations were considered viable if the response to three consecutive aliquots of ACh (10^{-6} M), added every 30 min into the organ bath, was constant (0.36 ± 0.08 cm). Samples not responding to exogenous ACh or giving an irregular response, were discarded.

In the first set of experiments, the response of equine jejunum to exogenous ACh, alone or after the incubation with muscarinic antagonists, was assessed. ACh was added cumulatively to the bath solution in 1-log unit increments of concentration (10^{-8}-10^{-2} M), in order to obtain concentration-response curves. Since the contractile effect of ACh was not reproducible, and the preparations became unresponsive after being exposed to maximum concentration, probably due to desensitisation phenomena, a single concentration-response curve of ACh was carried out in the same sample. For each experiment, four preparations of jejunal circular muscle were obtained from the same horse, of which one was used for constructing the concentration-response curve of the agonist alone (control curve) (Fig. 1A), while the others were incubated with a single concentration of the antagonist for 30 min, before the generation of ACh curve (Fig. 1B). When surmountable antagonism was obtained, i.e. when increasing concentrations of each antagonist induced a parallel and concentration-related rightward shift of ACh curve, without affecting maximum effect (E_{max}), the potency of the antagonist was calculated according to a previously described method (Arunlakshana and Schild, 1959). For each concentration of the antagonist, concentration ratios (CR) were calculated by dividing the concentration of the agonist giving 50% of maximum effect (EC_{50}) obtained in the presence of the antagonist by the EC_{50} calculated for the control curve. By plotting log [CR-1] against the -log [antagonist concentration], a linear regression was obtained for each antagonist; the intercept on X axis of this line, if the slope is not significantly different from

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unity, represents the pA_2 (-log K_B, K_B being the equilibrium constant of the antagonist), which is an index measure of the antagonist potency at the receptor.

In another set of experiments, Electrical Field Stimulation (EFS) was applied with a pair of coaxial platinum electrodes positioned along the longitudinal axis of the preparations, and used to deliver trains of square wave pulses (0.5 ms duration, 50 V amplitude) every 60 sec to the tissue at a frequency of 10 Hz (Basile, Milan, Italy). Under these conditions, depolarization of intrinsic nerve endings and neurotransmitter release were induced. For each experiment, the intensity was adjusted to a level giving 70-80% of the maximum tissue response (usually 250-300 mA), in order to be able to measure either the reduction and the increase of the contraction amplitude. The effect of each muscarinic antagonist on EFS-evoked contractions was assessed: drugs were added cumulatively to the bath solution in 1-log unit increments of concentration, in order to obtain concentration-response curves. The changes of EFS-evoked phasic contractions of the preparations induced by drugs were expressed as percentage of pre-drug amplitude of contractions (0.87 ± 0.11 cm), assumed as 100%. The potency of each antagonist was expressed by the concentration giving 50% of maximum inhibitory effect (IC_{50}) from individual fitted concentration-response curves, by using nonlinear regression with variable slope, and expressed with pKi value (-Log IC_{50}).

The effects of muscarinic antagonists on ACh concentration-response curves, and on EFS-induced motility were recorded with a pen-writing polygraph (Basile, Milan, Italy); all the calculations were performed by using a commercial software (GraphPad Prism ver. 6.0, GraphPad Inc., La Jolla, CA, USA). The data are expressed as mean ± SE of 8 experiments for each drug.

3. Results

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3.1 Effects of muscarinic antagonists on ACh concentration-response curves

Nonselective muscarinic antagonist, atropine (10^{-9}-10^{-7} M), antagonized the ACh-induced contractions in a surmountable and concentration-related fashion (pA_2 = 9.78 ± 0.21) (Fig. 2A). Pirenzepine, selective M_1 antagonist, (10^{-7}-10^{-5} M), and p-FHHSiD, selective M_3 antagonist, (10^{-7}-10^{-5} M), antagonized ACh-induced contractions in a concentration-related, competitive fashion (pA_2 = 7.14 ± 0.25 and 7.56 ± 0.17, respectively) (Fig. 2B and 2D). Methoctramine, M_2 selective antagonist, at 10^{-7} M was ineffective (not shown), and was able to antagonize ACh contraction at 10^{-6} M, while at a ten-fold higher concentration (10^{-5} M), it was surprisingly less effective, inducing only a minimal rightward shift of the ACh curve (Fig. 2C). However, methoctramine 10^{-6}-10^{-5} M was able to antagonize carbachol, a nonselective muscarinic agonist, in a concentration-dependent manner (pA_2 = 6.42 ± 0.23) (Fig. 3). The pA_2 values of all muscarinic antagonists are reported in Table 1.

3.2 Effects of muscarinic antagonists on EFS-evoked contractions

EFS evoked regular spikes of contraction overcoming spontaneous motility (Fig. 4). These contractions were abolished by TTX and ω-CTX GVIA (data not shown). Atropine (10^{-9}-10^{-5} M) reduced the amplitude of contractions in a concentration-dependent manner, reaching a maximal effect of (-45.64 ± 6.54%) (Fig 4); the remaining contractions were inhibited by neurokinin receptor block obtained by simultaneously adding into the organ bath L-732,138, GR159897 and SB218795 10^{-6} M, selective antagonists at NK_1, NK_2, and NK_3 receptor, respectively (Fig. 4). M_3 selective antagonist, p-FHHSiD (10^{-8}-10^{-5} M) was able to decrease EFS-induced contractions, showing a lower potency (pKi = 5.80 ± 0.13) compared to atropine (pKi = 7.16 ± 0.20) but greater efficacy (-63.75 ± 8.92%) (Fig. 5A). By contrast, M_1 selective antagonist, pirenzepine (10^{-8}-10^{-5} M) was scarcely effective in reducing jejunal contractions up to 10^{-5} M, reaching a maximal effect of -25.40 ± 8.57%, whereas M_2 selective block by methoctramine was ineffective up to 10^{-7} M, and

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induced an increase of phasic contractions at 10^{-6} M (+12.91 ± 15.39%) (Fig. 5A). Following the block of neurokinin receptors by concurrently adding into the organ bath NK_1, NK_2 and NK_3 receptor antagonists at 10^{-6} M, methoctramine-induced enhancement of contraction at the concentration of 10^{-6} M was abolished, and M_2 selective antagonist slightly decreased the contractions at 10^{-8}-10^{-6} M, while it was considerably more effective (-42.92 ± 14.45%) at the highest concentration (10^{-5} M) (Fig. 5B). The effect of pirenzepine on EFS-evoked contractions was not modified following neurokinin receptor block, while p-FHHSiD reached a greater maximal effect at 10^{-5} M (-88.00 ± 7.48%) (Fig. 5B).

4. Discussion

The effects of muscarinic antagonists on ACh concentration-response curves seem to confirm that M_3 receptors play a paramount role in the cholinergic contraction of circular smooth muscle in jejunum of horses, as observed in previous studies (Marti et al., 2005; Teixeira-Neto et al., 2012). Atropine, a nonselective muscarinic antagonist, competitively antagonized ACh contraction, showing that activation of muscarinic receptor is involved. Accordingly, pA_2 value of atropine in this study is consistent with what was found in other studies (Hulme et al., 1990; Pesic et al., 2009). However, when a single MR subtype was blocked by using selective antagonists, only M_1 and M_3 selective antagonist, respectively pirenzepine and p-FHHSiD, were able to competitively antagonize ACh-evoked contractions, causing a rightward shift of ACh curve, whereas M_2 selective antagonist, methoctramine, antagonized ACh effect in a concentration-unrelated manner. Calculated pA_2 for p-FHHSiD was 7.56 ± 0.17, which is in accordance with the value measured for M_3 receptor in previous studies (Lambrecht et al., 1988; Barocelli et al., 1993; Menozzi et al., 2014), and the pA_2 value for pirenzepine (7.14 ± 0.25) was equal to the one described in a previous study on isolated horse jejunum (Teixeira-Neto et al., 2012), and compatible with those calculated in the

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gastrointestinal tract of other species (Eglen et al., 1996). However, the affinity previously calculated for pirenzepine at M_1 receptor was 8.3, while it was 6.9 at M_3 (Hammer et al., 1980), thus suggesting that the antagonism exerted by pirenzepine against ACh observed in our study was probably due to an interaction with M_3 receptors. Methoctramine, at the concentration of 10^{-5} M, was surprisingly less active with respect to ten-fold lower concentration. The reasons behind the loss of efficacy of M_2 selective antagonist at the highest concentration remains unclear without further studies, but an inhibition of acetylcholinesterase (AChE), enhancing the binding of ACh to MRs, could be a possible explanation. Indeed, methoctramine was previously shown to be an AChE inhibitor (Melchiorre et al., 1998), and the hypothesis of a reduced degradation of ACh seems to be further supported by the effect of methoctramine on carbachol concentration-response curve observed in our study. Methoctramine was indeed able to antagonize carbachol, which is resistant to degradation by AChE, in a concentration-dependent fashion; the calculated pA_2 for methoctramine against carbachol was 6.42 ± 0.23, a value that is compatible with the known affinity of this compound for M_3 receptor, but indeed very far from the expected pA_2 for M_2 receptor (7.9) (Melchiorre et al., 1987). Collectively, the effects of muscarinic antagonists on ACh concentration-response curves seem to confirm the predominant importance of M_3 receptors in mediating the contraction of intestinal smooth muscle observed in most studies (Ehlert et al., 1997; Marti et al., 2005; Takeuchi et al., 2007; Teixeira-Neto et al., 2012). By contrast, a relevant role by M_1 or M_2 receptor subtypes in mediating the contractions induced by exogenous ACh in horse jejunum seems to be unlikely. This latter finding might seem in contrast with previous evidence of a contribution by M_2 receptors to cholinergic contraction of intestinal smooth muscle (Eglen, 2001), and with the prevalence of M_2 over M_3 receptors found in the small intestine of several species (Giraldo et al., 1987; Michel and Whiting, 1988; Candell et al., 1990). However, the negligible role of M_2 receptors in mediating the contraction induced by exogenous ACh can be explained by the fact that this muscarinic receptor subtype, by inhibiting cAMP synthesis, opposes to the relaxation of sympathetic origin (Kume and Kotlikoff, 1991; Kotlikoff et al., 1999) but is devoid of a direct

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contractile action on smooth muscle cells, and therefore its effect might not be evident in isolated jejunum, in absence of adrenergic stimulation.

The study of EFS-evoked motility of horse jejunum revealed that these contractions were only of partially cholinergic nature, as atropine, up to 10^{-5} M, was able to inhibit them only by -45.64%. The atropine-insensitive component of EFS-evoked contractions was prevented by neurokinin receptor block, thus showing that tachykinins, such as substance P and neurokinin A, are involved as excitatory Non-Adrenergic Non-Cholinergic (eNANC) neurotransmitters. Even though the mixed cholinergic/NANC nature of EFS-evoked contractions complicates the interpretation of the results, the block of different MR subtypes with selective antagonists yielded interesting results. p-FHHSiD was very close to atropine in the ability to inhibit the contractions, suggesting that ACh, released from neurons of myenteric plexus, induces the contraction of smooth muscle in equine jejunum by M_3 receptor activation. This finding confirms what was observed about ACh concentration-effect curves, and is in accordance with the prevalent role by M_3 receptor subtype in the contraction of small intestine in horses, as well as in other species, found in previous studies (Ehlert et al., 1997; Marti et al., 2005; Takeuchi et al., 2007; Teixeira-Neto et al., 2012).

Pirenzepine, selective M_1 antagonist, was scarcely effective in reducing the amplitude of EFS-induced contractions, suggesting a marginal importance, if any, of M_1 receptors. The selective block of M_2 receptors with methoctramine produced interesting results, as it was ineffective up to 10^{-7} M, whereas it enhanced the amplitude of contraction spikes at 10^{-6} M. This result might suggest an interaction with neuronal M_2 receptors. Indeed, the presence of presynaptic MRs, which inhibit neurotransmitter release, has already been demonstrated in the small intestine of several species (Kilbinger and Wessler, 1980; Coulson et al., 2002; Takeuchi et al., 2005), as well as in the airways of horses (Wang et al., 1995). However, since EFS-evoked contractions of horse jejunum were of mixed cholinergic/neurokininergic nature, and in order to better understand the functional role of different MR subtypes, the effects of muscarinic antagonists were assessed also after the block of neurokinin receptors. When all three neurokinin receptors were blocked, methoctramine did not

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increase the amplitude of contractions, thus suggesting that an interaction between M_2 receptors and NANC neurons might occur. It could be hypothesized that presynaptic M_2 receptors may act by inhibiting neurokinin release from NANC excitatory neurons. In accordance with this, previous studies showed that M_2 receptors negatively regulate CGRP-release in rat skin, inhibit nociception in cutaneous nerve endings of mice (Bernardini et al., 2001; Bernardini et al., 2002), and that substance P-mediated contraction of mouse ileum is inhibited via M_2-subtype muscarinic receptor activation (Takeuchi et al., 2007). Further studies with selective neurokinin receptor block would be of great interest in order to better clarify the interaction between M_2 muscarinic receptors and NANC innervation of horse small intestine, also because of the important role of neuropeptides, such as substance P or neurokinin A, in inflammation. The effect of pirenzepine on EFS-evoked contractions was not modified in a relevant degree in presence of neurokinin receptor block, whereas p-FHHSiD was more effective, reaching a maximum effect of -88.00%, thus providing further evidence that the contractions mediated by the release of endogenous ACh are mediated by the activation of receptors of the M_3 subtype.

The results of this ex vivo study may also be of clinical interest, since it seems that methoctramine, a selective M_2 receptor antagonist, is not affecting jejunal contractions mediated by ACh in a relevant degree, while enhancing the contractions of neurokininergic nature, and thus could represent a safer alternative to nonselective antimuscarinic drugs for the prevention of vagally-mediated bradycardia in horses, reducing the incidence of post-operative bowel hypo-motility. In accordance with such finding, it was previously observed that methoctramine increased heart rate and cardiac output, without delaying the recovery of normal intestinal motility in horses anesthetized with halothane and xylazine (Teixeira Neto et al., 2004). However, it is often difficult to correlate data obtained in isolated organs to those observed in vivo; indeed, in our experimental conditions, the possible influence of M_2 receptors on sympathetic control of smooth muscle motility in horse jejunum was not investigated, and, as it could represent a limitation of the study, will be the subject of further research.

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In conclusion, this study seems to confirm the paramount importance of M_3 receptor in mediating the contraction of horse jejunum evoked both by endogenous and exogenous ACh, while the role of M_1 receptor subtype seems to be negligible. As for M_2 receptor, a relevant contribution to cholinergic contractions in equine jejunum was not detected in our experimental conditions, whereas it was observed that M_2 receptors possess an inhibitory action on excitatory NANC system.

Acknowledgements

The authors wish to thank Dr Alessandro Cenci, and Dr Giuseppe Placenza for their precious assistance.

Source of funding

This study was supported by a local grant from the University of Parma (FIL 2014).

Authors' declaration of interest

No competing interests have been declared.

Ethical considerations

Tissues used in this study were obtained from a private abattoir. The owner of the abattoir has given the permission for the publication of the present data.

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Figure legends

Fig. 1. Original recordings of control ACh concentration-response curve (10^{-8} M-10^{-2} M) (A), and of ACh concentration-response curve after incubation with nonselective muscarinic antagonist, atropine 10^{-9} M (B).

Fig. 2. Effects of increasing concentrations of nonselective muscarinic antagonist, atropine (atr) (A), selective M_1 antagonist, pirenzepine (pir) (B), selective M_2 antagonist, methoctramine (met) (C), and selective M_3 antagonist, p-FHSSiD (D) on ACh concentration-response curves in smooth muscle of horse jejunum. All data represent mean ± SE from 8 experiments.

Fig. 3. Effects of increasing concentrations of selective M_2 antagonist, methoctramine (met), on carbachol concentration-response curve in smooth muscle of horse jejunum. All data represent mean ± SE from 8 experiments.

Fig. 4. Above: effect of increasing concentrations of nonselective muscarinic antagonist, atropine, on EFS-evoked contractions of horse jejunum. Below: effect of the simultaneous block of neurokinin receptors with 10^{-6} M L-732,138, GR159897, and SB218795 (selective NK_1, NK_2 and NK_3 receptor antagonists, respectively) on the atropine-insensitive component of EFS-evoked contractions; original recording. Data represent mean ± SE from 8 experiments.

Fig. 5. Effects of increasing concentrations of selective M_1 antagonist, pirenzepine, selective M_2 antagonist, methoctramine, and selective M_3 antagonist, pFHSSiD on EFS-evoked contractions of horse jejunum in absence (A), and in presence (B) of the simultaneous block of neurokinin receptors with 10^{-6} M L-732,138, GR159897, and SB218795 (selective NK_1, NK_2 and NK_3 receptor antagonists, respectively). Data represent mean ± SE from 8 experiments.

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Table 1
Calculated pA_2, slope, and R^2 values for muscarinic antagonists on ACh concentration-response curve in jejunal smooth muscle of horses. Values represent mean ± SE.

Antagonist (receptor)	pA_2	slope	R^2
Atropine (nonselective)	9.78 ± 0.21	0.81 ± 0.12	0.96
Pirenzepine (M_1 selective)	7.14 ± 0.25	0.87 ± 0.11	0.94
Methoctramine (M_2 selective)	6.42 ± 0.23 (*vs* carbachol)	0.78 ± 0.23	0.96
p-FHHSiD (M_3 selective)	7.56 ± 0.17	0.83 ± 0.17	0.96

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Fig. 1

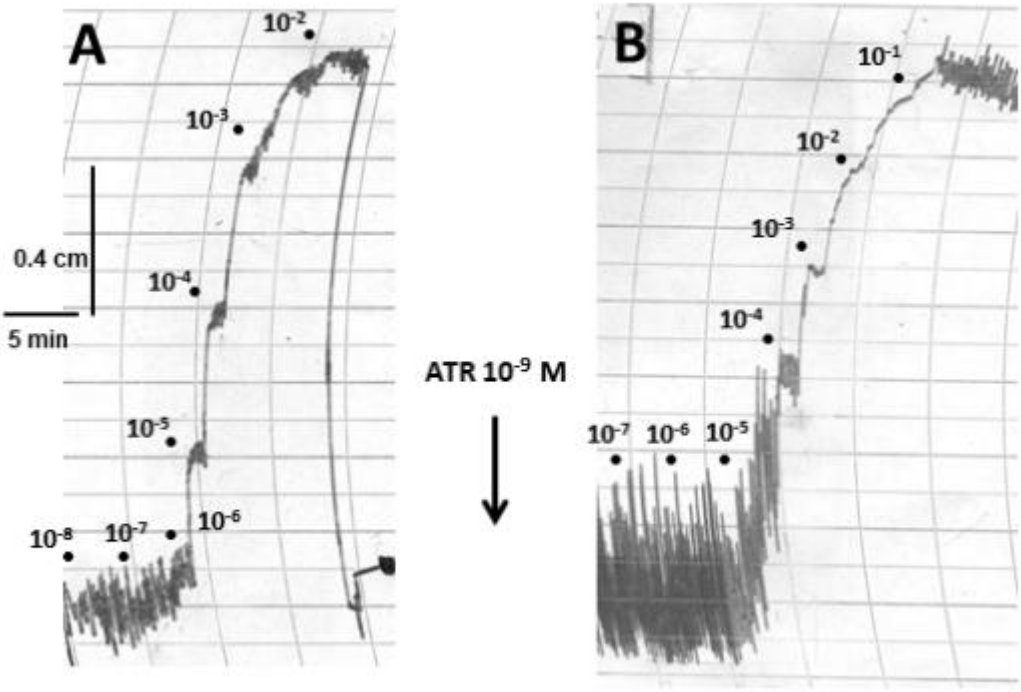

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Fig. 2

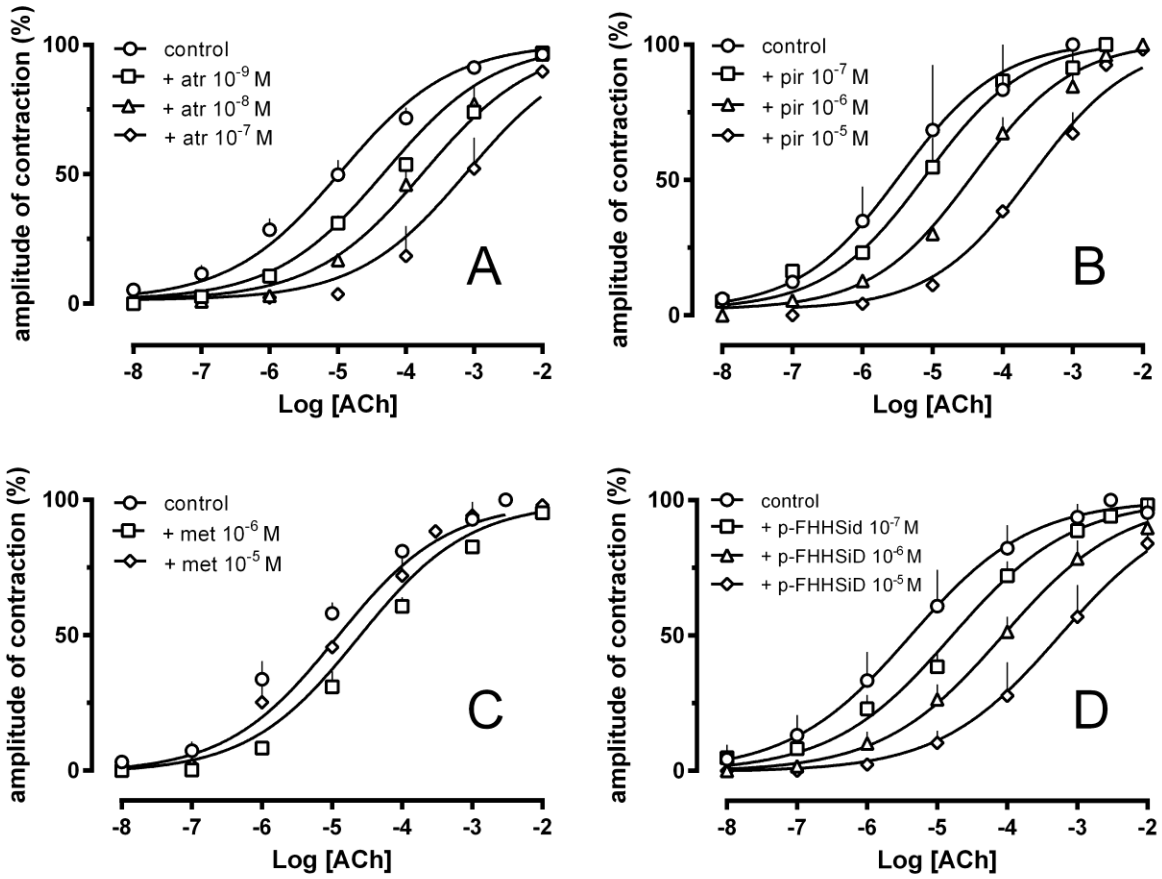

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Fig. 3

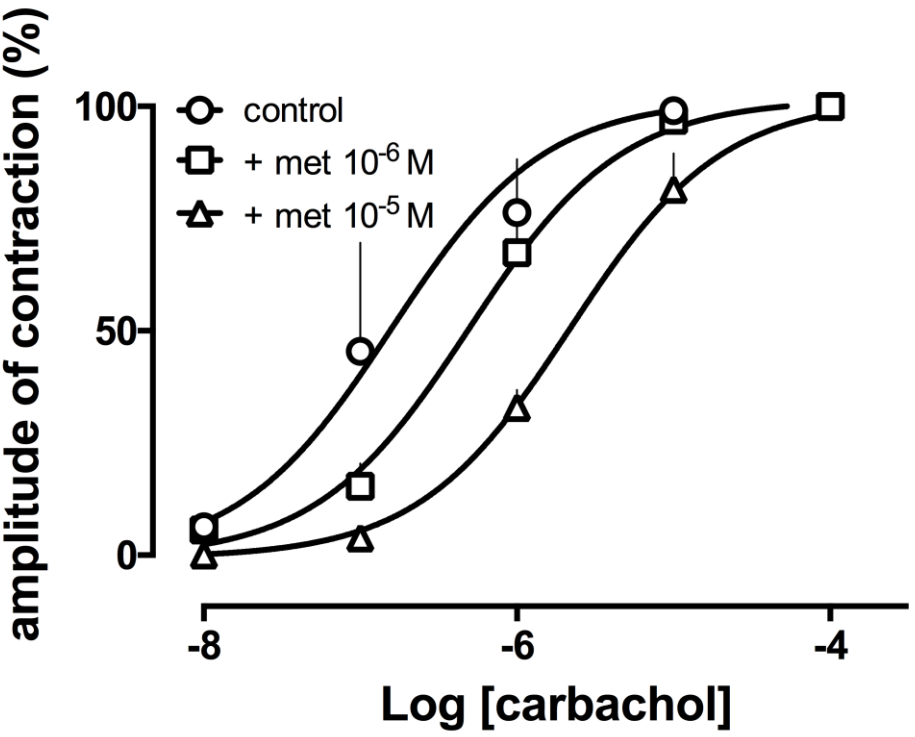

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Fig. 4

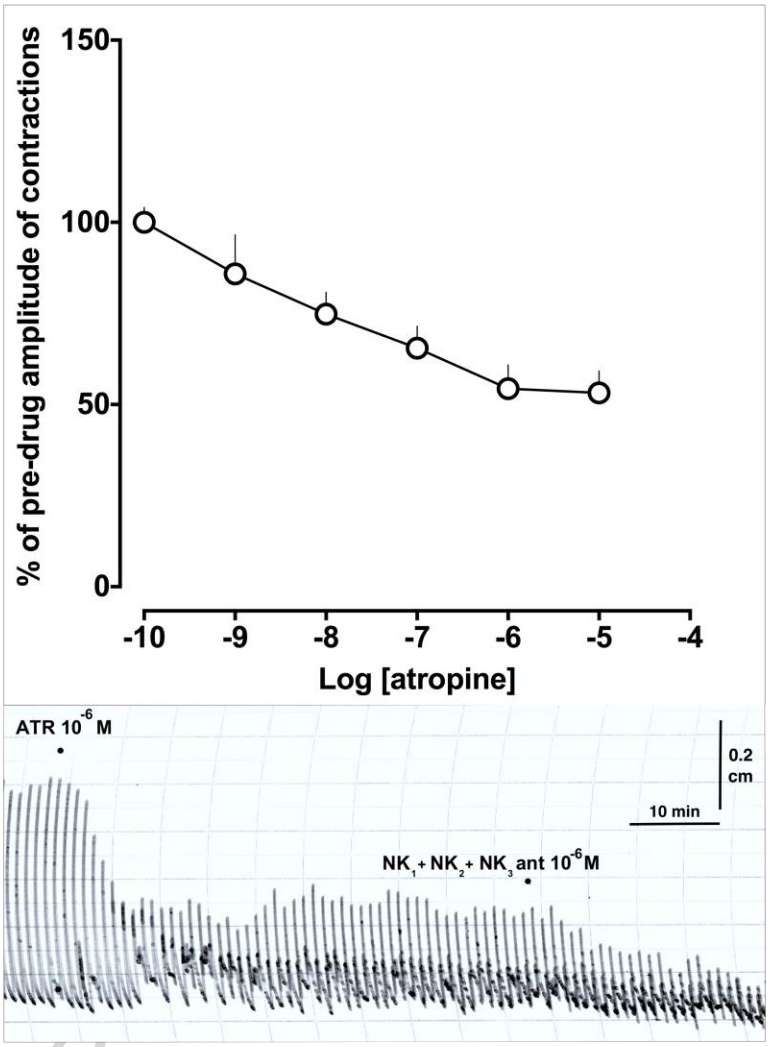

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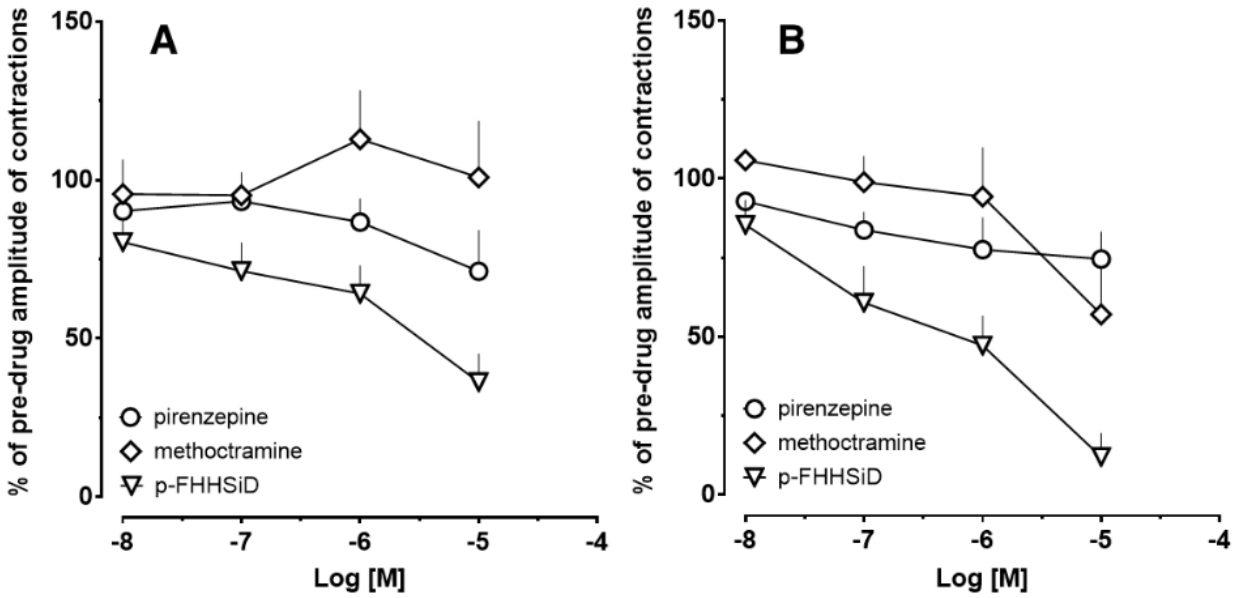
Fig. 5



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Highlights

- The role of muscarinic receptors in horse jejunum motility was investigated in vitro
- The effects of nonselective and selective M1, M2 and M3 antagonists were assessed
- Drug-induced modifications of ACh- and EFS-evoked contractions were evaluated
- Cholinergic contraction of horse jejunum is mediated by M3 receptors
- M2 receptors have an inhibitory activity on contractions of neurokininergic nature

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