® Postepy Hig Med Dosw (online), 2011; 65: 478-481
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2011.06.07
Accepted: 2011.07.07
Published: 2011.08.03

Modulating activity of M_1 receptor to the reaction of ileal smooth muscle*

Modulujące działanie receptora M_1 na reakcję mięśniówki gładkiej jelita krętego

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Summary

Background: The subject of the study was determination of the effect of drugs on ileal smooth muscle contraction induced by activation of M_1 type muscarinic receptors. Drugs that have an effect on muscarinic receptors are divided to agonists, with close ties to the receptor and high internal activity and antagonists, with no internal activity. Conducted experiments tested interactions between a broad-spectrum agonist of muscarinic receptors, carbachol and a selective muscarinic receptor antagonist of M_1 type, pirenzepine.

Material/Methods: Testing was conducted on tissues isolated from rat's intestine. Male Wistar rats with weight between 220 g and 360 g were anesthetized by intraperitoneal injection of urethane (120 mg/kg). Concentration-effect curves were determined with the use of cumulated concentration method, in accordance with the van Rossum method (1963) in Kenakin modification (2006).

Results: The purpose of the study was determination of concentration-effect curves for carbachol. This curve was compared with the curve of receptor occupation depending on concentration of this drug. Based on concentration-effect curves, the average value of EC_{50} was calculated for carbachol, amounting to 2.44×10^{-6} [M/l].

Conclusions: The results confirmed that atropine is effective in stopping contractions caused by carbachol, meeting the conditions of competitive antagonists. Atropine caused the shift of curves for carbachol to the right. Pirenzepine, selectively blocking muscarinic receptors of M_1 type gave similar results. It was proved that in the preparation of gastric fundus smooth muscle, M_1 type receptors occur not only presynaptically, but also postsynaptically.

Key words: smooth muscle • ileum • muscarinic receptors • atropine • pirenzepine

Streszczenie

Wstęp: Przedmiotem pracy jest określenie wpływu leków na skurcz mięśniówki gładkiej jelita krętego wyzwalany aktywacją receptorów muskarynowych typu M_1. Leki wpływające na receptory muskarynowe dzielą się na agonistów, leki o dużym powinowactwie do receptora i dużej aktywności wewnętrznej oraz antagonistów, niemających aktywności wewnętrznej. W przeprowadzonych doświadczeniach badano interakcje między szerokospektralnym agonistą receptorów muskarynowych – karbacholem a selektywnym antagonistą receptorów muskarynowych M:1 – pirenzepiną.

* Publication funded by the European Union under the European Social Fund – Project "Development Programme of Collegium Medicum UMK".

Materiał/Metody: Badania przeprowadzono na tkankach wyizolowanych z jelita szczura. Samce szczurów szczepu Wistar o masie 220–360 g usypiano uretanem (120 mg/kgm.c.) wstrzykiwanym dootrzewnowo. Krzywe stężenie – efekt wyznaczano metodą stężeń kumulowanych, zgodnie z metodą van Rossuma (1963) w modyfikacji Kenakin (2006).

Wyniki: Celem pracy było wyznaczenie krzywych stężenie – efekt dla karbacholu. Krzywa ta została porównana z krzywą zajęcia receptorów w zależności od stężenia tego leku. Na podstawie uzyskanych krzywych stężenie – efekt obliczono średnią wartość EC_{50} dla karbacholu, która wyniosła $2{,}44\times10^{-6}$ [M/l].

Wnioski: Uzyskane wyniki potwierdziły, że atropina skutecznie hamuje skurcze wywołane karbacholem, spełnia więc warunki stawiane antagonistom kompetycyjnym. Atropina powodowała przesunięcie krzywych dla karbacholu w prawo. Pirenzepina, selektywnie blokująca receptory muskarynowe typu M_1 dała podobne rezultaty. Dowiedziono, że w preparacie mięśniówki gładkiej dna żołądka, receptory typu M_1 występują nie tylko presynaptycznie, lecz także postsynaptycznie.

Słowa kluczowe: **mięśniówka gładka • jelito kręte • receptory muskarynowe • atropina • pirenzepina**

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=954785

Word count: 1104
Tables: 1
Figures: 4
References: 16

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Introduction

The subject of the study was determination of the effect of drugs on ileal smooth muscle contraction induced by activation of M_1 type muscarinic receptors. Drugs that have an effect on muscarinic receptors are divided to agonists, with close ties to the receptor and high internal activity and antagonists, with no internal activity [1,4]. Conducted experiments tested interactions between a broad-spectrum agonist of muscarinic receptors, carbachol and a selective muscarinic receptor antagonist of M_1 type, pirenzepine.

In accordance with the receptor theory, the condition for activity of drug is its reaction with the cell protein (receptor), resulting in changes in its activity. Receptor means unique places of bonding of drug with the cell, which intermediate in activity of the drug. They can be found on the surface of the cell or inside cytoplasm [9]. Muscarinic receptors M_1 are frequently called neuronal receptors. They occur on parietal cells, in the central and peripheral nervous system. M_1 receptors release stimulation effects; one of the examples is slow muscarinic stimulation depending on acetylcholine in sympathetic ganglions and central neurons [9,11,12]. This simulation is caused by drop in conduction for potassium ions, resulting in depolarization of the neuronal membrane. Muscarinic receptors M_1 also stimulate secretion of hydrochloric acid in the stomach after vagus nerve stimulation [2,9,15].

Material and Methods

Testing was conducted on tissues isolated from rat's intestine. Male Wistar rats with weight between 220 g and 360 g were anesthetized by intraperitoneal injection of urethane (120 mg/kg). The intestine was dissected under anesthetic; after dissection it was cut out and placed in a dish for insulated organs with 20 ml in capacity, filled with oxidized Krebs fluid. Content of Krebs fluid: 71.8 mM NaCl, 4.7 mM KCl, 1.7 mM $CaCl_2$, 28.4 mM $NaHCO_3$, 11.7 mM glucose, 2.4 mM $MgSO_4$, 1.2 mM KH_2PO_4. A series of testing with inadequate blood supply was conducted under controlled conditions. The intestine was dissected and arteries were pressed with Klem clamps for a period of 30 minutes, in order to induce ischemia; after that, Klem clamps were removed and the intestine was dissected after 90 minutes.

In addition to that testing, another one was performed; after 30 minutes from closing blood vessels vascularizing the intestine, Klem tools were used to dissect the organ. After that, it was placed in oxidized Krebs fluid, followed by analysis of reaction. Testing was carried out 30 minutes after closing dishes and 90 minutes after reperfusion of the dish. Preparations were added to the dish in the amount between 0.1 ml and 0.3 ml.

Concentration-effect curves for tested agonists and antagonists were determined with the use of the Van Rosum method – concentrations increasing every 0.5 log. Based on these concentration-effect curves, constants were determined for tested agonists, specifying activity of a given preparation, i.e. EC_{50}. The control curve of EC_{50} value was determined based on 25 curves and under controlled conditions it amounts to $2.44(\pm0.11)\times10^{-7}$; it can serve as basis for recreation of the theoretical curve.

Concentration-effect curves were determined with the use of cumulated concentration method, in accordance with the van Rossum method (1963) in Kenakin modification (2006).

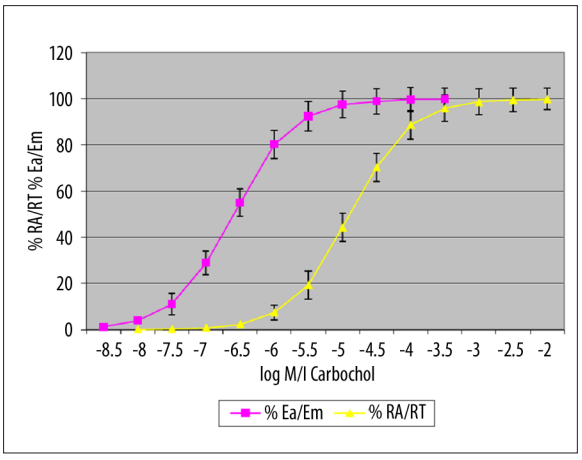

Fig. 1. Concentration-effect curve for carbachol and RA/RT dependence curve for carbachol. Constant K_A 1.26(±0.09)×10^{-5}. Points marked on the curve present average values and SE for n=9

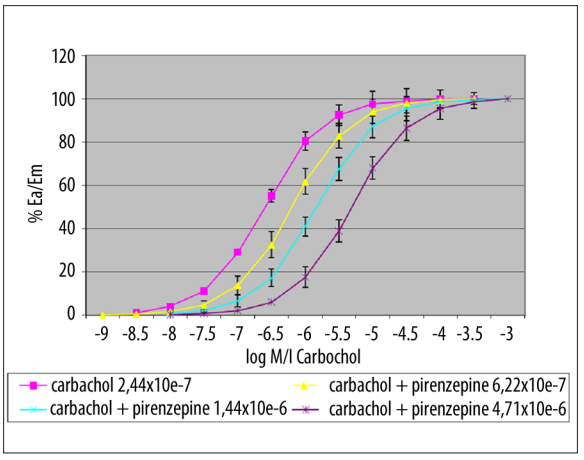

Fig. 2. Influence of increasing concentrations (from 10^{-7} to 10^{-6} [M/l]) of pirenzepine on concentration-effect curve for carbachol. Points marked on the curve present average values and SE ± for n =9

Table 1. Influence of carbachol on the reaction of ileal smooth muscle contraction before and after the use of increasing concentrations of pirenzepine

Drug	n	EC_{50} [M/l]	K_A [M/l]
Carbachol	9	2.44×10^{-7}	1.26×10^{-5}
Carbachol + Pirenzepine 10^{-7}	9	6.22×10^{-7}	1.37×10^{-5}
Carbachol+ Pirenzepine 3×10^{-7}	9	1.44×10^{-6}	1.46×10^{-5}
Carbachol+ Pirenzepine 10^{-6}	9	4.71×10^{-6}	1.51×10^{-5}

Results

Carbachol, in the range of concentrations between 10^{-8} and 10^{-3}, causes ileal smooth muscle contraction that depends on concentration. Concentration-effect curves for carbachol were used for determination of the average value of EC_{50}, which amounts to 2.44(±0.11)×10^{-7} for n=9. Results are presented on Fig. 1 and in Table 1.

A series of experiments made with dibenamine, an irreversible antagonist, was used for determination of Ka, a dissociation constant, which amounts to 1.26(±0.09)×10^{-5} for n=9. This constant was used for determination of a curve, presenting dependence between%RA/RT and concentration of carbachol (Fig. 1).

Pirenzepine, a relatively selective receptor antagonist of M_1 type, in the range of concentrations between 10^{-7} and 10^{-6} [M/l], causes concentration-dependent shift of concentration-effect curve for carbachol to the right, maintaining maximum reaction. Based on these curves, EC_{50} values were determined in the presence of increasing concentrations of pirenzepine. Results are shown in Table 1. The average for n=9 is shown by concentration-effect curve for carbachol in the presence of pirenzepine in concentrations between 10^{-7} and 10^{-6}, presented on Fig. 2.

The use of pirenzepine, a competitive receptor agonist M_1, causes shift of concentration-effect curve (for carbachol) to the right, maintaining maximum reaction.

According to analysis of curves, we can deduce that pirenzepine meets the conditions posed to competitive antagonists, whereas the average value of IC_{50} determined for pirenzepine amounts to 1.89×10^{-9} [M/l]. Fig. 3 presents concentration-effect curve for antagonistic activity of pirenzepine in relation to a non-selective muscarinic receptor agonist and carbachol.

Atropine, as a non-selective muscarinic receptor antagonist, causes concentration-dependent shift of concentration-effect curve (for carbachol) to the right, maintaining maximum reaction. According to the determined curve, we can deduce that atropine meets the conditions posed to competitive antagonists.

Discussion

According to testing conducted to date, we can confirm that rat's ileum contains muscarinic receptors. Existence of these receptors acknowledges antagonistic activity of atropine, a non-selective muscarinic receptor antagonist [13,14,15]. According to presented data, pirenzepine, a relatively selective receptor antagonist shows activity similar to atropine. According to analysis of curves and EC_{50} values for carbachol determined during experiments, we can deduce that pirenzepine meets the conditions of competitive antagonism in relation to carbachol, a non-selective muscarinic receptor agonist. The IC_{50} value for pirenzepine, determined during experiments, amounts to 1.89(±0.16)×10^{-8} [M/l]. During testing conducted in the laboratory on an insulated gastric fundus with darifenacin, it was determined that M_3 type receptor occurs in smooth muscle and intermediates in contraction release. IC_{50} value for darifenacin amounts to 3.89(±0.12)×10^{-8} [M/l]. In addition, according to testing conducted to date, it was determined that

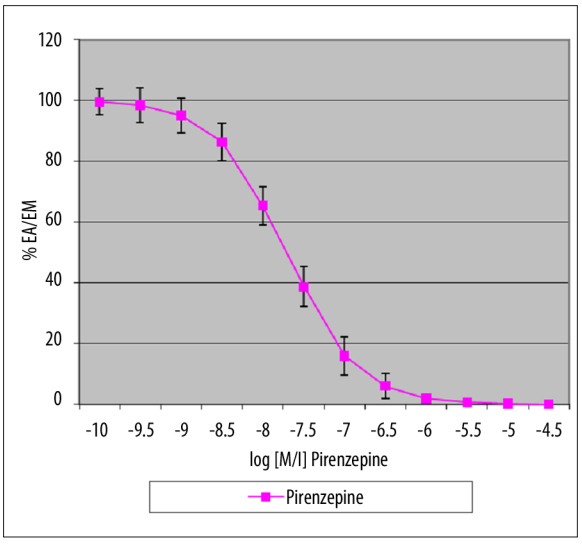

Fig.3. Presentation of concentration-effect curve determined for antagonistic activity of pirenzepine on ileal smooth muscle. The average value of IC_{50} pirenzepine for n=9 amounts to $1.89(\pm0.16)\times10^{-8}$ [M/l]

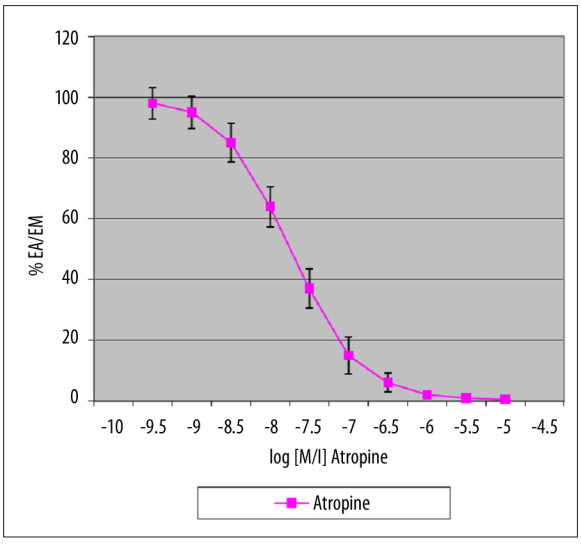

Fig. 4. Presentation of concentration-effect curve determined for antagonistic activity of atropine on ileal smooth muscle. The average value of IC_{50} atropine for n=9 amounts to $1.76(\pm0.08)\times10^{-8}$ [M/l]

M_1 type muscarinic receptors occur mainly on ends of the parasympathetic nervous system and fulfill a function modulating secretion of acetylcholine [3,10,16]. Activation of these receptors causes release of acetylcholine from ends of the parasympathetic nervous system. During testing, it was determined that M_1 type receptor occurs in smooth muscle and intermediates in contraction release [8,11,12].

Conclusions

- Carbachol in concentrations between 10^{-7} and 10^{-6} [M/l] causes concentration-dependent isometric ileal smooth muscle contraction.
- Atropine, a non-selective muscarinic receptor antagonist, meets the conditions of competitive antagonists.
- Pirenzepine, a relatively selective muscarinic receptor antagonist of M_1 type, breaks contraction induced by carbachol and meets the conditions posed to competitive antagonists.
- The results suggest occurrence of M_1 type receptors in rat's ileum.

Acknowledgment

I would like to express my gratitude to Professor Leszek Szadujkis-Szadurski for his kindness, understanding, valuable instructions and assistance in analysis of results and writing of my dissertation.

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The authors have no potential conflicts of interest to declare.