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Role of M1 receptor in regulation of gastric fundus smooth muscle contraction

Rola receptora M1 w regulacji mięśniówki gładkiej dna żołądka

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Summary

Background: The subject of this study is determination of the influence of drugs on gastric fundus smooth muscle contraction induced by activation of muscarinic receptors M1. Experiments tested interactions between a receptor agonist, carbachol and muscarinic receptor antagonists, atropine and pirenzepine.

Material/Methods: Testing was conducted on tissues isolated from rat's stomach. Male Wistar rats with weight between 220 g and 360 g were anesthetized by intraperitoneal injection of urethane (120 mg/kg). The stomach was dissected, and later the gastric fundus was isolated. Tissue was placed in a dish for insulated organs with 20 ml in capacity, filled with Krebs fluid. Results contained in the study are average values ± SE. In order to determine statistical significance, the principles of receptor theory were used (Kenakin modification).

Results: According to tests, carbachol, in concentrations ranging between 10^{-8} M to 10^{-4} M, in a dosage-dependent way induces gastric fundus smooth muscle contraction. Presented results indicate that carbachol meets the conditions posed to full agonists. On the other hand, atropine, a non-selective muscarinic receptor antagonist, causes a concentration-dependent shift of concentration-effect curve (for carbachol) to the right, maintaining maximum reaction. According to analysis of the curve determined, we can deduce that atropine meets the conditions posed to competitive antagonists. The use of pirenzepine, a competitive receptor agonist M1, causes shift of concentration-effect curve (for carbachol) to the right, maintaining maximum reaction.

Conclusions: From the testing conducted on the preparation of the gastric fundus we can deduce that atropine causes shift of concentration-effect curves for carbachol to the right. A similar effect is released by pirenzepine, selectively blocking muscarinic receptors of M1 type. The results indicate that in the preparation of the gastric fundus smooth muscle, M1 type receptors occur also postsynaptically.

Key words: **smooth muscle contraction • gastric fundus • carbachol • pirenzepine**

Streszczenie

Wstęp: Przedmiotem pracy jest określenie wpływu leków na skurcz mięśniówki gładkiej dna żołądka wyzwalany aktywacją receptorów muskarynowych M1. W przeprowadzonych doświadczeniach badane były interakcje między agonistą receptorów muskarynowych – karbacholem a antagonistami receptorów muskarynowych: atropiną oraz pirenzepiną.

Wyniki: Z przeprowadzonych badań wynika, że karbachol, w stężeniu 10^{-8}–10^{-4} M, w sposób zależny od dawki wyzwala skurcz mięśniówki gładkiej dna żołądka. Przedstawione wyniki wskazują, że karbachol spełnia warunki stawiane pełnym agonistom.

Atropina natomiast, nieselektywny antagonista receptorów muskarynowych, powoduje przesunięcie krzywej stężenie-efekt (dla karbacholu) w sposób zależny od stężenia w prawo, z zachowaniem maksymalnej reakcji. Na podstawie wyznaczonej krzywej można stwierdzić, że atropina spełnia wszystkie warunki stawiane antagonistom kompetycyjnym.

Zastosowanie pirenzepiny, agonisty kompetycyjnego receptorów M1, powoduje przesunięcie krzywej stężenie-efekt (dla karbacholu) w prawo, z zachowaniem maksymalnej reakcji.

Wnioski: Z przeprowadzonych badań na preparacie dna żołądka wynika, że atropina powoduje przesunięcie krzywych stężenie-efekt w prawo (dla karbacholu). Podobny efekt wyzwala pirenzepina selektywnie blokująca receptory muskarynowe typu M1. Uzyskane wyniki wskazują, iż w preparacie mięśniówki gładkiej dna żołądka, receptory typu M1 występują także postsynaptycznie.

Słowa kluczowe: **skurcz mięśniówki gładkiej • dno żołądka • karbachol • pirenzepina**

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Introduction

The subject of this study is determination of the influence of calmodulin and calcium on gastric fundus smooth muscle contraction. Regulation of smooth muscle contraction is important for the course of many essential physiological mechanisms such as motor functions of the alimentary canal, including the gastric fundus. The main mechanism causing smooth muscle contraction is an increase of the intracellular concentration of calcium ions, although sensitivity of the contractile apparatus can be subject to many modifications as a result of activity of specific agonists [2,3,8,10]. Reaction of contraction induced by serotonin can be modified by cGMP, cAMP, as well as factors blocking the release of calcium from the endoplasmic reticulum. In addition, contraction can be caused by an inflow of calcium ions from extracellular fluids to cytoplasm by canals located in the cell membrane. Diastole, however, is related to, among others, activation of guanylate cyclase receptors (CG) [1,5,9].

The study analyzed interaction between serotonin agonists, serotonin inducing smooth muscle contraction and cyclical nucleotide – 8Br cGMP, YC-1, ODQ (guanylate cyclase inhibitor) and flunarizine [4,6,7].

Material and Methods

Testing was conducted on male Wistar rats with weight between 220 g and 360 g. Animals were anesthetized with urethane. The stomach was dissected, and later the gastric fundus was isolated. Tissue was placed in a dish for insulated organs with 20 ml in capacity, filled with Krebs fluid.

Regardless of traditional Krebs fluid, tests were also using Krebs fluid deprived of calcium ions. Tested preparations were added to the dish in the amount between 0.1 and 0.3 ml. The purpose of testing conducted in fluid without calcium ions was determination of intracellular role of calcium in released contraction. This process can take place through two mechanisms. The first one consists in activation of IP_3 receptors, whereas the second one involves activation of ryanodine receptors with the use of ryanodine or caffeine. In testing conducted in calcium fluid, intracellular calcium pool was eliminated by using Ca-ATPase inhibitor, cyclopiazonic acid or Thapsigargin.

An inflow of calcium to the cell takes place by passive diffusion, in accordance with concentration gradient, through two types of channels, VOC – Voltage Operated Calcium Channel (controlled by potential on the cell membrane) and ROC – Receptor Operating Calcium Channel (controlled by receptors). Opening of VOC channels and closing for a flow of calcium ions depend on the volume of electric potential flowing to them, namely on the level of their polarization. A phenomenon of opening of calcium channels as a result of their depolarization is called electromechanical coupling. Opening of ROC channels depends on specific receptors located on these channels, meaning mechanism called pharmacomechanical coupling.

Based on concentration-effect curves determined for tested agonists, constants were marked, specifying activity of the preparations used, including:

- EC_{50} (concentration releasing 50% of maximum reaction),
- dissociation constant of a given drug acting with Ka receptor.

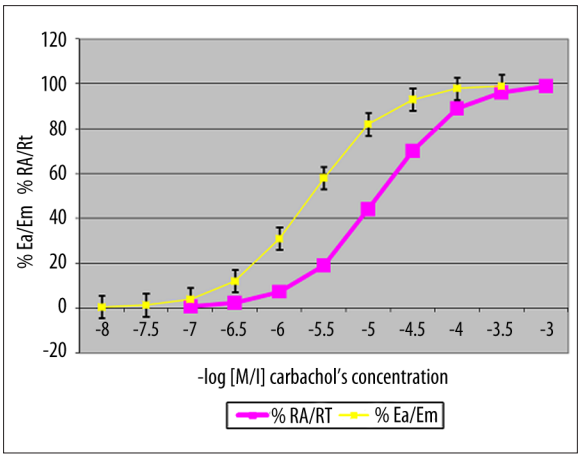

Fig. 1. Concentration-effect curve determined for contractile activity of carbachol on the gastric fundus smooth muscle and%RA/Rt/ carbachol concentration dependence curve

Concentration-effect curves for tested agonists and antagonists were determined with the use of the van Rossum method (increasing concentrations, every half of logarithm).

Results

Carbachol, in the range of concentrations between 10^{-8} M and 10^{-4} M, in a dosage-dependent way induces gastric fundus smooth muscle contraction (Fig. 1). The average value of EC50 for carbachol amounts to 2.17×10^{-6} M/for n=25. The chart also shows Ka constant for carbachol, determined based on 25 concentration-effect curves after irreversible inactivation of reserve receptors by phenoxybenzamine 10^{-7} M/l. The average value of Ka constant for carbachol amounts to 1.26×10^{-5}.M/l. Fig. 1 presents dependence curve between% RA/Rt and carbachol concentration. Comparing concentration-effect curve with RA/Rt/carbachol concentration dependence curve, we can confirm that the curve presenting% of occupied receptors is shifted to the right. Presented results indicate that carbachol meets the conditions posed to full agonists. In addition, presented data confirms that there is a reserve receptor pool in the tested preparation of the gastric fundus.

On the other hand, atropine, a non-selective muscarinic receptor antagonist, causes a concentration-dependent shift of concentration-effect curve (for carbachol) to the right, maintaining maximum reaction. According to analysis of the curve determined, we can deduce that atropine meets the conditions posed to competitive antagonists. Fig. 2 presents concentration-effect dependence curve for antagonistic activity of atropine on gastric smooth muscle. Based on curves determined, the average IC_{50} value for atropine amounts to 1.76×10^{-8} M/l.

The use of pirenzepine, a competitive receptor agonist M1, causes shift of concentration-effect curve (for carbachol) to the right, maintaining maximum reaction. According to analysis of the curve determined, we can deduce that pirenzepine meets the conditions posed to competitive antagonists, and the average IC_{50} value for pirenzepine amounts to 4.89×10–9 M/l. Fig. 3 presents concentration-effect curve for antagonistic activity of pirenzepine in relation of a non-selective muscarinic receptor agonist and carbachol.

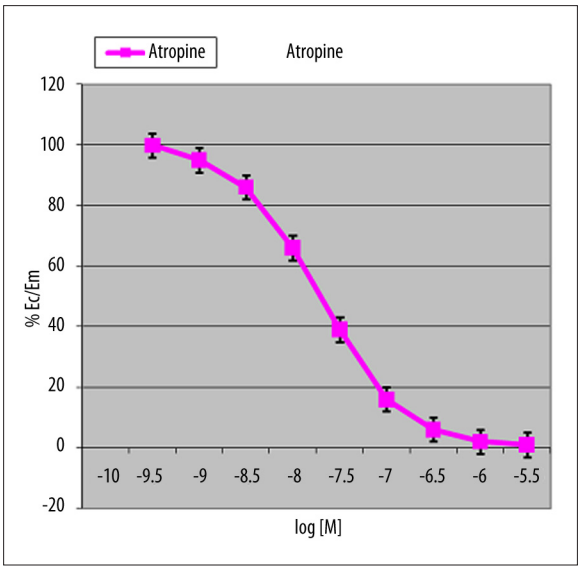

Fig. 2. Presentation of concentration-effect curve determined for antagonistic activity of atropine on gastric smooth muscle. The average IC_{50} value for atropine for n=9 amounts to 1.76 (±0.08)×10^{-8} M/l

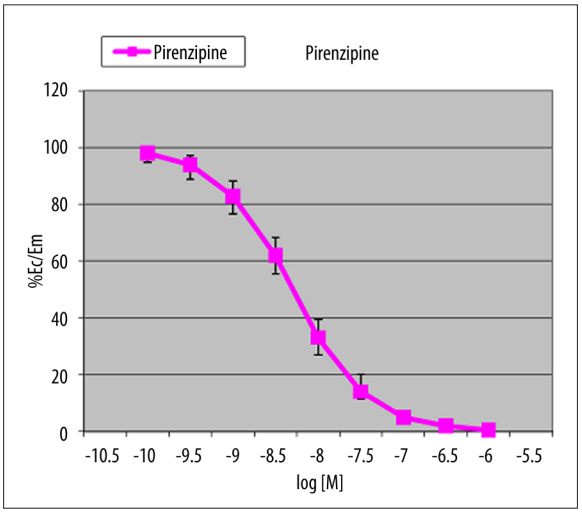

Fig. 3. Presentation of concentration-effect curve for activity of pirenzepine on gastric fundus smooth muscle. The average IC_{50} value for pirenzepine for n=9 amounts to 4.89 (±0.08)×10^{-8} M/l

Discussion

According to the analysis conducted, in compliance with the principles of the receptor theory, we can deduce that atropine, a non-selective muscarinic receptor antagonist, stops effectively contractions induced by carbachol. It should be remembered that carbachol is also a non-selective antagonist.

Analyzing the curves, in compliance with the principles of the receptor theory, we can deduce that atropine meets the conditions posed to competitive antagonist, and IC_{50} for this agonist amounts to 1.91×10^{-8} M/l.

Results obtained from testing with the use of atropine were compared with the activity of pirenzepine, a selective antagonist blocking M1 type muscarinic receptors. It

is known that receptors occur primarily on synaptic ends. In addition, they induce contractions. The results indicate that M1 receptors occur not only presynaptically, but also postsynaptically.

Conclusions

1. In testing conducted on preparation of the gastric fundus, atropine causes shift of concentration-effect curves (for carbachol) to the right.
2. A similar effect is induced by pirenzepine, selectively blocking M1 type muscarinic receptors.
3. The results indicate that in the preparation of the gastric fundus smooth muscle, M1 type receptors occur also postsynaptically.

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The authors have no potential conflicts of interest to declare.