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Role of calcium and calmodulin in reaction of gastric fundus contraction

Rola wapnia i kalmoduliny w reakcji skurczu dna żołądka

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Summary

Background:

The subject of this study is determination of the influence of calmodulin and calcium on gastric fundus smooth muscle contraction. During experiments, the author tested the influence of a serotonin receptor agonist, serotonin (5-HT), causing smooth muscle contraction.

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 \pm SE. In order to determine statistical significance, the principles of receptor theory were used (Kenakin modification).

Results:

According to conducted tests, we can deduce that 8 Br cGMP stops the reaction of gastric fundus smooth muscle contraction induced by serotonin. The use of 8Br-cGMP in the range of concentrations between 10 and 300 μ M leads to reduction of maximum effect from 100% to 46%. Similar changes were obtained after the use of guanylate cyclase activator (CG) – YC-1. Curves for the contractile activity of serotonin along with an increase of concentration YC-1 are shifted to the right, and the maximum effect of reaction decreases. Increasing concentrations of flunarizine, a calmodulin antagonist, in a concentration-dependent way blocks binding between calcium and calmodulin, and at the same time leads to the shift of concentration-effect curves for serotonin to the right and a decrease of maximum reaction.

Increasing concentrations of ODQ, a guanylate cyclase inhibitor lead to statistically significant shift of the curves to the left, decrease of EC_{50} value and simultaneous increase of maximum reaction to serotonin.

Conclusions:

According to conducted testing, serotonin causes gastric fundus smooth muscle contraction dependent on concentration. Reaction of contraction induced by serotonin is stopped by a calmodulin antagonist, flunarizine. In addition, experiments confirmed participation of cyclical nucleotides in blocking reaction of gastric fundus contraction.

Key words:

smooth muscle contraction • gastric fundus • calmodulin • calcium

Streszczenie

Wstęp:

Przedmiotem pracy jest określenie wpływu kalmoduliny oraz wapnia na skurcz mięśniówki gładkiej dna żołądka. W przeprowadzonych doświadczeniach badano wpływ agonisty receptorów serotoninowych – serotoniny (5-HT), wywołującej skurcz mięśni gładkich.

Materiał/Metody:

Badania przeprowadzono na tkankach wyizolowanych z żołądka szczura. Samce szczurów szczepu Wistar o masie 220–360 g usypiano uretanem (120 mg/kg m.c.) wstrzykiwanym dootrzewnowo. Preparowano żołądek, po czym izolowano dno żołądka. Krzywe stężenie– efekt wyznaczano metodą stężeń kumulowanych, zgodnie z metodą van Rossuma (1963) w modyfikacji Kenakin (2006).

Wyniki:

Z przeprowadzonych badań wynika, że 8 Br cGMP hamuje reakcję skurczu mięśniówki gładkiej żołądka wyzwalaną serotoniną. Zastosowanie 8Br-cGMP w zakresie stężeń 10–300 μM powoduje obniżenie maksymalnego efektu 100–46%. Podobne zmiany uzyskano po zastosowaniu aktywatora cyklicznej guanylanowej (CG) – YC-1. Krzywe dla kurczącego działania serotoniny wraz ze wzrostem stężenia YC-1 ulegają przesunięciu w prawo, a efekt maksymalny reakcji obniża się. Wzrastające stężenia flunarizyny – antagonisty kalmoduliny, w sposób zależny od stężenia hamuje wiązanie wapnia z kalmoduliną i jednocześnie powoduje przemieszczenie krzywych stężenie – efekt dla serotoniny w prawo oraz obniżenie maksymalnej reakcji.

Wzrastające stężenia ODQ – inhibitora cyklicznej guanylanowej w sposób istotny statystycznie powodują przesunięcie krzywych w lewo, obniżenie wartości EC₅₀ i jednocześnie podwyższenie maksymalnej reakcji na serotoninę.

Wnioski:

Z przeprowadzonych badań wynika, że serotonina powoduje skurcz mięśniówki gładkiej żołądka w sposób zależny od stężenia. Reakcja skurczu wywołanego serotoniną hamowana jest przez antagonistę kalmoduliny – flunarizynę. Doświadczenia potwierdziły ponadto udział cyklicznych nukleotydów w hamowaniu reakcji skurczu dna żołądka.

Słowa kluczowe:

skurcz mięśniówki gładkiej • dno żołądka • kalmodulina • wapń

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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 [7,8]. 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) [1,2,6,7,12,13,14].

The study analyzed interaction between serotonin agonists, serotonin inducing smooth muscle contraction and cyclic nucleotide – 8Br cGMP, YC-1, ODQ (guanylate cyclase inhibitor) and flunarizine [3,4,5,8,9,10,11,15].

MATERIAL AND 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 [9,17].

Regardless of traditional Krebs fluid, tests were also using Krebs fluid deprived of calcium ions. The purpose of testing conducted in fluid without calcium ions was determination of intracellular role of calcium in released contraction [8,16].

Based on determined concentration-effect curves, constants were marked, specifying activity of the used preparations: EC₅₀ and a dissociation constant of a given drug acting with receptor – Ka. Concentration-effect curves for tested agonists and antagonists were determined with the use of the Van Rosum method. The control curve of EC₅₀ value was determined based on 25 curves and under controlled conditions it amounts to 2.17×10⁻⁶; it can serve as basis for recreation of the theoretical curve. Results contained in the study are average values ± SE. In order to determine statistical significance, the principles of receptor theory were used (Kenakin modification) [13].



Table 1. Determined values $EC_{50} \pm SE$ and N for substances used during experiments conducted under controlled conditions and conditions of gastric fundus smooth muscle contraction

Drug	N	$EC_{50} (\pm SE)$
5-HT	9	3.2×10^{-7} [M/l] (± 0.06)
5-HT+8BrGMP/10 uM	9	6.7×10^{-7} [M/l] (± 0.27)
5-HT+8BrGMP/30 uM	9	1.26×10^{-6} [M/l] (± 0.17)
5-HT+8BrGMP/100 uM	9	3.72×10^{-6} [M/l] (± 0.23)
5-HT	9	3.2×10^{-7} [M/l] (± 0.06)
5-HT+YC-1/1 uM	9	7.6×10^{-7} [M/l] (± 0.06)
5-HT+ YC-1/3 uM	9	2.17×10^{-6} [M/l] (± 0.11)
5-HT +YC-1/10 uM	9	6.41×10^{-6} [M/l] (± 0.27)
5-HT	9	3.2×10^{-7} [M/l] (± 0.06)
5-HT +ODQ/10 uM	9	1.17×10^{-6} [M/l] (± 0.23)
5-HT +ODQ/30 uM	9	6.61×10^{-8} [M/l] (± 0.16)
5-HT +ODQ/100 uM	9	3.79×10^{-8} [M/l] (± 0.14)
5-HT+Flunarizine /1 uM	9	4.22×10^{-8} [M/l] (± 0.06)
5-HT+Flunarizine /3uM	9	7.11×10^{-7} [M/l] (± 0.22)
5-HT+Flunarizine/10uM	9	2.97×10^{-6} [M/l] (± 0.17)

The following reagents were used in testing: Serotonin (3-[2-Aminoethyl]-5-hydroxyindol), 8 Br cGMP (Beringher), YC-1 (Sigma) and ODQ (Sigma)

The experiments were carried out using of Krebs' fluid (normal) – PSS - composition: NaCl (71.8 mM/L), KCl (4.7 mM/L), $CaCl_2$ (1.7 mM/L), $NaHCO_3$ (28.4 mM/L), $MgSO_4$ (2.4 mM/L), KH_2PO_4 (1.2 mM/L), glucose (11.1 mM/L) with the addition of EGTA (30 μ M/L).

RESULTS

Serotonin (5HT) with concentration amounting to 3.2×10^{-7} (± 0.06) M/l releases concentration-dependent gastric fundus smooth muscle contraction. The use of 8Br-cGMP in the range of concentrations of 10, 30, 100 μ M, respectively, causes decrease of maximum effect from 100% to 46%, with simultaneous shift of concentration-effect curves to the right. As a result of this activity, the value of EC_{50} for 5HT increases from 3.2×10^{-7} to 3.72×10^{-6} (± 0.23) μ M/l, which means that the value of EC_{50} increases approximately twelvefold.

Just like in the case of using 8Br cGMP, the use of YC-1 in concentrations: 1 μ M/l, 3 μ M/l and 10 μ M/l, causes decrease of maximum effect from 100% to 38%, shift of concentration-effect curves to the right, an increase of EC_{50} value, and decrease of maximum effect of reaction. Therefore, EC_{50} value increased approximately elevenfold.

The author also analyzed activity of ODQ (10 μ M, 30 μ M, 100 μ M) on contracted gastric fundus 5-HT (Fig.). EC_{50} for serotonin after the use of increasing ODQ concentrations decreases respectively to the value EC_{50} : 1.17×10^{-6} [M/l], (± 0.23), 6.61×10^{-8} [M/l], (± 0.16) and 3.79×10^{-8} [M/l]

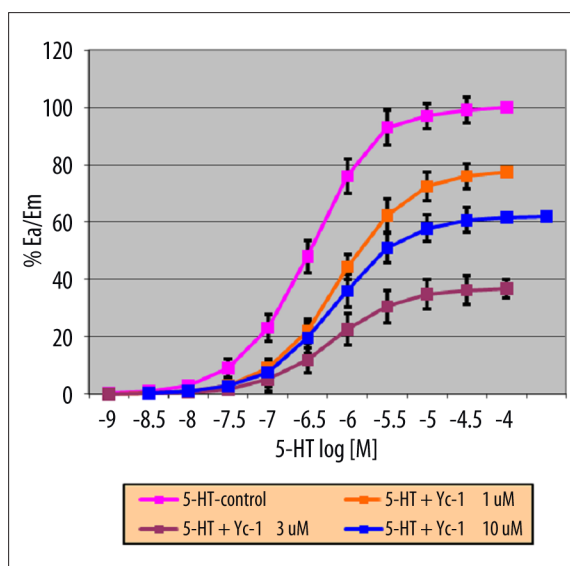


Fig. 1. Concentration-effect curve for 5HT before and after the use of 8Br cGMP. Concentration-effect curve for 5HT after the use of various concentrations 8Br cGMP shifts to the right, as a result of which EC_{50} increases to 6.7 μ M/l ± 0.17 , 1.2 μ M/l ± 0.17 , 3.72 μ M/l ± 0.17 , for n=9

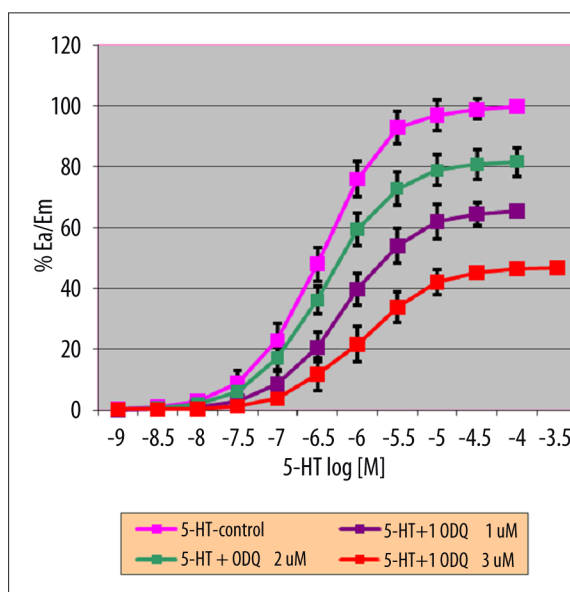


Fig. 2. Concentration-effect curve determined for contractile activity of 5-HT on the gastric fundus, determined before and after the use of YC-1; (EC_{50} values for consecutive concentrations YC-1 – Tab. 1)

(± 0.14). In addition, after the use of ODQ, maximum reaction of Em value was increasing, respectively.

The use of flunarizine with concentrations: 1 μ M, 3 μ M and 10 μ M, causes decrease of maximum effect from 100% to 30%, shift of concentration-effect curves to the right, and an increase of EC_{50} value to: 4.22×10^{-8} [M/l] (± 0.06), 7.11×10^{-7} [M/l] (± 0.22) and 2.97×10^{-6} [M/l] (± 0.17).

DISCUSSION

Calcium ions are essential for smooth muscle contractions. These contractions can be regulated through many various

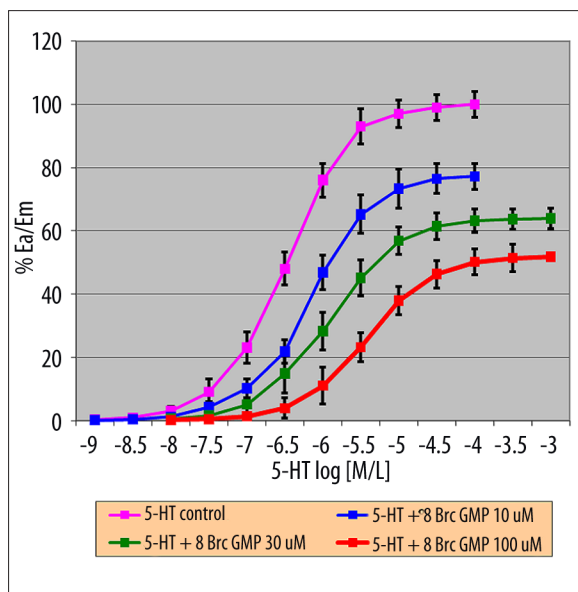


Fig. 3. Concentration-effect curve determined for contractile activity of 5-HT on the gastric fundus, determined before and after the activity of ODQ (EC_{50} values for consecutive concentrations ODQ – Tab. 1)

substances stimulating the calcium ions' release from the cellular storages. Cyclic nucleotide analogs, such as cAMP and cGMP, which through the protein kinase regulate the function of the ion channels allowing smooth muscle contractions. Experiments conducted in earlier years showed the blocking influence of cGMP analogs on the reaction of smooth muscle contractions, including gastric fundus smooth muscle contractions. The author also tested the influence of a serotonin receptor agonist, serotonin. According to tests, 8 Br cGMP stops the reaction of gastric fundus smooth muscle contraction induced by serotonin. Under the influence of this nucleotide, concentration-effect curves for serotonin shift to the right with simultaneous decrease of the effect of maximum reaction.

Similar changes in the shape of concentration-effect curves were obtained after the use of guanylate cyclase activator (CG)- YC-1. Curves for the contractile activity of serotonin along with an increase of YC-1 concentration shift to the right with simultaneous decrease of the effect of maximum reaction [16,17].

Increasing concentrations of ODQ, a guanylate cyclase inhibitor, have a statistically significant effect on the shift of curves to the left, decrease of EC_{50} value and an increased maximum reaction to serotonin.

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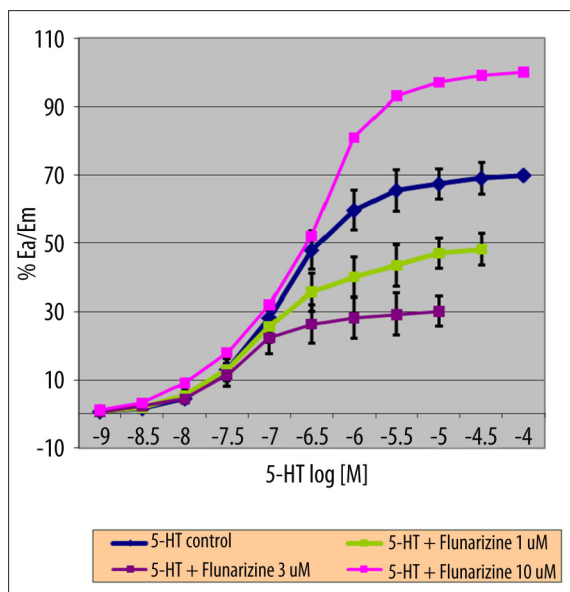


Fig. 4. Concentration-effect curve determined for contractile activity of 5-HT on the gastric fundus, determined before and after the activity of flunarizine (EC_{50} values for consecutive concentrations of flunarizine – Tab. 1)

On the other hand, experiments conducted with flunarizine showed that this calmodulin antagonist in a concentration-dependent way blocks binding of calcium with calmodulin, causing simultaneous shift of concentration-effect curves for serotonin to the right and decrease of maximum reaction.

CONCLUSIONS

1. In testing conducted on preparation of the gastric fundus, serotonin causes concentration-dependent smooth muscle contraction.
2. Reaction of contraction induced by serotonin is blocked by flunarizine, a calmodulin antagonist.
3. YC-1 – CG activator and 8 BrcGMP cause similar changes in reaction of the gastric fundus to serotonin.
4. Confirmation of participation of CG and cGMP in regulation of smooth muscle contraction induced by serotonin was obtained by using ODQ inhibitor.
5. Confirmation of participation of CG and cGMP in regulation of smooth muscle contraction induced by carbachol was obtained by using ODQ inhibitor.

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