

ANTAGONIZING OF PROSTAGLANDIN EFFECTS THROUGH STOMATOSTATIN. STUDIES IN VITRO

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Abstract

This paper is focused on the effect of somatostatin on the bovine, human purified erythrocyte carbonic anhydrase (CA) and on the one in the gastric mucosa, and after the activation of the enzyme with pentagastrin.

Key words: Somatostatin, Carbonic Anhydrase, pentagastrin

INTRODUCTION

The carbonic anhydrase (CA), an enzyme widely distributed in the body and in the parietal cell, is activated by the pentagastrin and it is inhibited by the somatostatin.

This action was highlighted *in vitro* and *in vivo* both for the lab animals and for the humans.

The action of somatostatin on the elimination of gastrin, basic and stimulated through arginine, was studied *in vitro* by HAYES and his collaborators, on fragments of antral rat wall. These fragments were placed in a room, being dripped at an alkaline pH, varying between 7.35 and 7.45, at the temperature of 37°C (Tulassay Z, Gupta R, et.al 1989).

We studied the effect of a pretreatment through somatostatin on the release of gastrin, induced by the arginine (Torres AJ, Landa I, Hernandez F, et al. 1986). The gastrin was measured through radioimmunological dosing with rabbit antibodies and human antigastrin I (Gjorup J, Roikjaer O, Anderson B, et al. 1992).

The somatostatin does not have any action on the basic gastrinemia's level. Through stimulation with arginine and the concomitant administration of somatostatin, the gastrin secretion does not modify (Cerragio F, Bertini G, et al. 1989).

The technique *in vitro* allows the elimination of *feed-back* between the parietal and antral cells, resulting in an action through an intermediary mechanism (Tulassay Z, Gupta R, et.al 1989). The impossibility to demonstrate a somatostatin action on the basic gastrin level is explained

through the fact that the plasma is present under several forms and they are not subject to the same regulating mechanism.

In the following paper, we studied the somatostatin effect on bovine, human purified erythrocyte CA and on the one in the gastric mucosa, and after the activation of the enzyme with pentagastrin, performing determinations of the relationship dose – response and kinetic studies (Kayasseh L, Gyr K, et.al. 1980).

It is compared with the cimetidin, antagonist of H_2 - histamine receptors, it inhibits the acid gastric secretion and it blocks the secreting effects of histamine and gastrin.

MATERIAL AND METHODS

Somatostatin, pentagastrin, bovine erythrocyte purified CA II, HEPES buffer, 4 –nitrophenilacetate, were obtained from SERVA, Heidelberg; cimetidin from SKF England; 4 – nitrophenol, Tris, imidazole from E.MERCK, Darmstadt; synthetic analogue of somatostatin – octeroid (Somatostatin) from SANDOZ AG, Nurnberg; kit for determining the CO_2 concentration from GILFORD SYSTEM (Oberlin, Ohio, USA).

The determinations of human erythrocyte CA were performed from the blood of ill individuals without digestive disorders, and for the CA from the gastric mucosa it was performed through the isolation of parietal cells from dock stomach (Corragio F, Bertini G, et al. 1989).

The determination of CA kinetic activity was performed through several methods: one based on the CO_2 hydrating reaction, using the diffusimetric technique of alternating pH and the second was based on the hydrolyze reaction of 4 – nitrophenilacetate (Shroder T, Millard RW, et.al., 1988).

Within the kinetic studies, we determined the maximum velocity of reaction (V_{max}) and the Michaelis constants (K_m) for pentagastrin, somatostatin.

The initial velocity within the CO_2 hydrating reaction, as well as the reaction dose – response was measured with a Hi – TECH SF – 51 MX device (we used 4 – nitrophenol as pH indicator and Trisimidazol buffer at $pH = 9.5$ at $T = 25^{\circ}C$). These determinations were also performed in the absence of the buffer at $pH = 7.5$.

The data were manipulated through a compatible RCD – IBM computer, provided with DAS -50 interface (Omega, England) and kinetic software I.S. -1 (RKBIN).

The initial CO_2 concentration was enzymatically determined on an IMPACT 400 (Gilford, USA) analyzer.

The initial velocity for the hydrolyze reaction of 4 – nitrophenilacetate spectrophomerically was determined at 348 nm with the spectrophometer

Gilford Respose. We worked in HEPES buffer at pH = 7.5 and K2S04 for ionic strength.

The kinetic data were manipulated according to the Michaelis – Menten equation, using the Linearveaver – Burk linearization.

RESULTS DISCUSSION

The pentagastrin activates the bovine erythrocyte purified CA at concentrations between $10^{10} - 10^{-4}$ (Figure 1).

At equimolecular concentrations, the somatostatin antagonizes the activator effect of pentagastrin (Jenkins SA, Kynaston H, et al. 1992).

Compared to somatostatin, the cimetidin does not modify the CA basic activity or the pentagastrin activator effect (Magnussen I, Ihre T, et al. 1985)

Similar results with the one obtained on bovine erythrocyte purified CA II were also obtained on human erythrocyte enzyme and on CA from the isolated parietal cells from a dog's stomach (Antonioli A, Gandolfo M, , et al. 1986).

The kinetic parameters obtained from the determinations and presented in tables 1, 2, indicate a non – competitive interaction both between CA and pentagastrin (as activator) and between CA and somatostatin (as inhibitor).

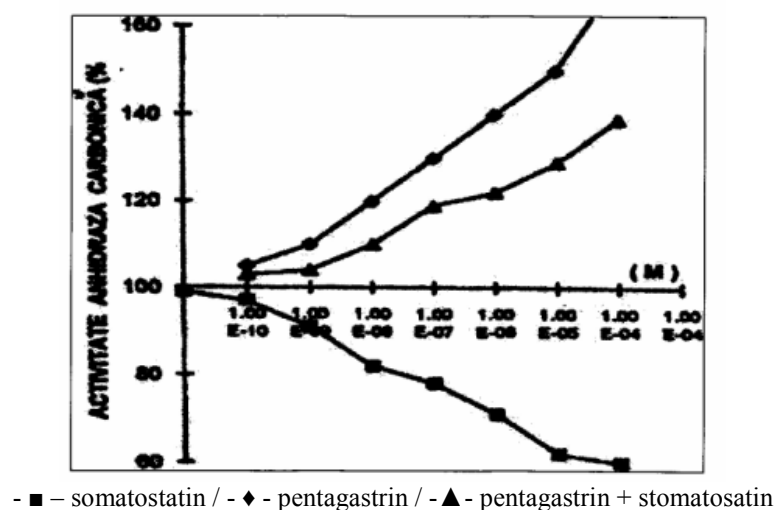


Figure 1. The effects of pentagastrin, somatostatin and the association of pentagastrin and somatostatin on the bovine erythrocyte purified Ca II. CA activity in the absence of activator or inhibitor is 100%.

Table 1.

Kinetic data for CA, pentagastrin (P), somatostatin (S) in the CO₂ hydrating reaction, pH = 7.5; T = 25⁰; CA concentration = 6.6 x 10⁻⁸ M

System	Vmax (mM * s ⁻¹)	K ^a m (mM)
CA	2.3 ± 0.03	8.90 ± 0.2
CA +P (10 ⁻⁵ M)	3.00 ± 0.03	8.93 ± 0.3
CA + S (10 ⁻⁵ M)	1.91 ± 0.02	8.85 ± 0.2
CA + P +S	2.87 ± 0.02	8.87 ± 0.3

Table 2.

Kinetic data for CA, pentagastrin (P), somatostatin (S) in the hydrolyze reaction of 4 - nitrophenilacetate, pH = 7.4; T = 25⁰; CA concentration = 2.29 x 10⁻⁶ M

System	Vmax (mM * s ⁻¹)	K ^a m (mM)
CA	1.70 ± 0.022	1.98 ± 0.3
CA +P (10 ⁻⁶ M)	2.12 ± 0.020	2.01 ± 0.2
CA + S (10 ⁻⁶ M)	1.35 ± 0.016	1.99 ± 0.2
CA + P +S	1.97 ± 0.020	1.97 ± 0.3

The decrease of CA basic activity and the antagonizing of the pentagastrin activator effect by the somatostatin could lead to new data in order to explain the mechanism of acid gastric secretion.

CONCLUSIONS

The similarities between the response of the parietal cell and CA to various activators and inhibitors lead us to presume the existence of some receptors involved in the regulation of acid gastric secretion.

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