Calcium, Carbonic Anhydrase and Gastric Acid Secretion

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Summary
Previous data concerning the action of calcium (Ca) on gastric acid secretion (GAS) indicated that calcium ions increase GAS elicited by gastrin released through a vagal mechanism, and also by a direct effect on parietal cells. Our research showed that the stimulating effect of calcium on gastric acid secretion can be antagonized by verapamil administration, which reduces gastric acid secretion. In the present study we followed the effect induced by administration of calcium and Ca-chelating agents (disodium EDTA) on gastric acid secretion and on carbonic anhydrase (CA) activity. We selected two groups of healthy volunteers: Group I (n=21) received a single i.v. dose of CaCl2 (15 mg/kg b.w.), whereas Group II (n=22) received a single i.v. dose of disodium EDTA (5 mg/kg b.w.). We determined blood calcium before and after treatment, gastric acid secretion at 2 hours, erythrocyte CA II activity, and CA IV activity in membrane parietal cells, which were isolated from gastric mucosa obtained by endoscopic biopsy. Assessment of carbonic anhydrase activity was achieved by the stopped-flow method. In Group I calcium administration increased blood calcium, HCl output, CA II and CA IV activity as compared to initial values. In Group II, disodium EDTA reduced blood calcium, HCl output, CA II and CA IV activity as compared to initial values. The results demonstrated that increased blood calcium and GAS values after calcium administration correlated with the increase of erythrocyte CA II and parietal cell CA IV activity, while disodium EDTA induced a reversed process. Our results also show that cytosolic CA II and membrane CA IV values are sensitive to calcium changes and they directly depend on these levels. Our data suggest that intra- and extracellular pH changes induced by carbonic anhydrase might account for the modulation of the physiological and pathological secretory processes in the organism.

Key words
Calcium • Disodium EDTA • Carbonic anhydrase • Gastric acid secretion

Introduction
Sidney Ringer published the first report relating cellular function with Ca2+ in 1883. He demonstrated that Ca2+ was necessary for normal regular contractions of the isolated frog heart. Following this landmark study, Ca2+ became an essential component of physiological saline solutions.

The first data concerning the action of calcium on gastric acid secretion (GAS) were published in 1927. Donegan and Spiro (1960) proved that calcium ions stimulate gastric acid secretion. Since then many articles have been published and they clearly demonstrated that
the administration of calcium in various forms increases gastric acid secretion. Reeder et al. (1970) and Grossman (1974) stated that stimulation of gastric acid secretion by calcium salts in the stomach releases gastrin. Muñell and Sachs (1984) assumed that calcium ion-induced stimulation was mediated by a vagal mechanism, because this stimulating effect can be abolished by atropine and magnesium.

Several research workers, e.g. Schratchard (1973) and Holtermuller (1974), proposed that calcium ions directly activate parietal cells. Calcium causes a release of antral gastrin, but this effect is not indispensable, since stimulation of gastric acid secretion does occur even after antrectomy (Konturek 1981).

Our previous work showed that administration of calcium both local, on the gastric mucosa, and systemic, by i.v. infusion, stimulates carbonic anhydrase (CA) activity in the gastric mucosa and red cells (Puscas et al. 1978). The stimulating effect of calcium on gastric acid secretion can be antagonized by inhibitors of calcium channels (verapamil). Our previous studies had shown that verapamil reduced gastric acid secretion in parallel with the inhibition of gastric mucosal carbonic anhydrase (Puscas et al. 1984). The same studies pointed out that i.v. administration of disodium EDTA strongly reduces gastric acid secretion (Puscas et al. 1978).

As far as the physiological role of carbonic anhydrase isozymes is concerned our studies indicated that CA I is involved in the vascular changes (Puscas and Coltau 1995a, Puscas et al. 1997), whereas CA II and CA IV are isozymes involved in the secretory processes (Puscas et al. 1999a). We have also proven that gastric CA IV is different from renal CA IV (Puscas et al. 1999b). Thus, unlike renal CA IV the gastric mucosa CA IV is activated by histamine, gastrin and acetylcholine, having a major role in the mechanism of gastric acid secretion (Puscas 1998a).

The same studies have also proved that CA I and CA II were activated by nonsteroidal anti-inflammatory drugs (NSAIDs) (Puscas et al. 1996). Moreover, vasodilating prostaglandins inhibited carbonic anhydrase while vasoconstrictive ones activated the enzyme (Puscas and Coltau 1995b).

However, our studies proved that carbonic anhydrase is not a mere catalyst of CO₂ hydration, but it plays a more complex role in regulating the vascular and secretory tonus. Other studies performed by us revealed a large number of endogenous and exogenous CA I, CA II and CA IV activators/inhibitors which enhance or reduce the activity of these isozymes affecting intracellular and extracellular pH. Our results led to the conclusion that through the pH changes induced by carbonic anhydrase activators and inhibitors, this enzyme may be considered a modulator of physiological and pathological processes in the organism (Puscas 1998b).

In present report we studied the effect induced by administration of calcium and Ca-chelating agents (disodium EDTA) on the activity of carbonic anhydrase isozymes II and IV and upon gastric acid secretion.

**Methods**

The study was conducted according to the Helsinki Declaration as modified by the 21st World Medical Assembly. A written formal consent was obtained from each patient. We selected two groups of healthy volunteers, aged 28-45, weighing 70-85 kg, as follows:

- **Group I** (n=21) received a single i.v. dose of CaCl₂, 15 mg/kg b.w.;
- **Group II** (n=22) received a single i.v. dose of 5 mg/kg b.w. disodium EDTA.

We determined blood calcium before and after treatment, gastric acid secretion at 2 hours after treatment, erythrocyte CA II, and parietal cell membrane CA IV activity which was isolated from the gastric mucosa obtained by endoscopic biopsy.

We determined CA IV activity by separation from parietal cells according to Maren et al. (1993). Red cell CA II activity was separated from red cell CA I according to the test with nicotinates (Puscas et al. 1999c). This test relies on the selective inhibition of CA I activity. In the first step, we assayed the total carbonic anhydrase activity. Methyl nicotinate added in the concentration of 5x10⁻⁴ M completely inhibited CA I activity. The remaining carbonic anhydrase activity in red blood cells represents CA II activity.

The carbonic anhydrase activity was assessed using the stopped-flow method (Khalifah 1971). This method consists in measuring the enzymatic activity of CO₂ hydration and is based on the colorimetric determination of the rate of pH change. The time in which the pH of the reagent mixture decreases from its initial value of 7.5 to its final value of 6.5 is measured. The follow-up of the reaction is achieved spectrophotometrically at 400 nm wavelength, using a rapid kinetic spectrophotometer HI-TECH SF-51MX (England), equipped with a mixing unit and a system of
two syringes which supply the reagents. The signal transmitted by the photomultiplier from the mixing chamber is received and visualized by a computer equipped with a mathematical coprocessor and a kinetic software package RKBIN IS1.

Reagents used
- p-nitrophenol - the color indicator at a concentration of 0.2 mM; pH=7.5; temperature 20-25 °C.
- HEPES buffer at concentration of 20 mM; pH=7.5; temperature 20-25 °C.
- the CO₂ solution at a concentration of 15 mM (as substrate) which was obtained by bubbling CO₂ in bidistilled water to saturation.
- Na₂SO₄ at a concentration of 0.1 M was used to maintain constant ionic strength.

Activity of carbonic anhydrase is obtained by the formula

\[ A = \frac{(T_0 - T)}{T} \text{[EU/ml]} \]

where \( T_0 \) represents the uncatalyzed reaction time, and \( T \) represents the catalyzed reaction time (in the presence of CA I, CA II gastric mucosa CA IV).

Statistics
To determine whether CA II and CA IV activity and HCl output were affected by calcium and EDTA, a repeated-measure ANOVA was performed. Comparisons between the treatments were made using the Neuman-Keuls multiple-comparison test. A paired t-test was used to compare initial values with the steady-state for a given treatment. Probabilities of \( p<0.05 \) were considered significant.

![Fig. 1. Blood calcium values after i.v. administration of CaCl₂ (Group I) and EDTA (Group II). Data are means ± S.E.M.; n=21-22: * significant differences (p<0.05) as compared with values before administration (paired t-test).](image1)

![Fig. 2. Basal acid output changes after i.v. administration of CaCl₂ (Group I) and EDTA (Group II). Data are means ± S.E.M.; n=21-22: * significant differences (p<0.05) as compared with values before administration (paired t-test).](image2)
Results

In Group I, calcium administration increased blood calcium (Fig. 1), HCl output (Fig. 2), CA II and CA IV activity (Fig. 3) as compared to initial values.

In Group II, disodium EDTA administration reduced blood calcium (Fig. 1), HCl output (Fig. 2), CA II and CA IV activity (Fig. 3) as compared to the initial values.

Discussion

Our results have shown that i.v. administration of calcium in man increased the levels of blood calcium which correlated with the increase of carbonic anhydrase activity and a parallel increase of gastric acid secretion. Administration of Ca-chelating drugs, such as disodium EDTA, reduced blood calcium levels along with a reduction of carbonic anhydrase activity and potent inhibition of gastric acid secretion. Our results suggest that the physiological or pathological changes in blood calcium concentration might influence both red cell and gastric mucosa carbonic anhydrase and bone tissue carbonic anhydrase activity. Thus, the values of GAS could be influenced by blood calcium levels. Nevertheless, our previous studies have shown that verapamil decreased red cell carbonic anhydrase activity, in parallel with GAS reduction but without significant changes in blood calcium levels (Puscas et al. 1984).

Considering the connection between parathyroid activity, blood calcium levels and the changes of carbonic anhydrase activity induced by changes in blood calcium levels, it may be assumed that parathyroid glands contribute to the regulation of carbonic anhydrase activity in the organism by means of blood calcium.

Ca\(^{2+}\) is an ubiquitous intracellular regulator of cellular function. Ca\(^{2+}\) levels are controlled by various channels, exchangers and pumps located in plasmalemmal and internal membranes. Cell stimulation causes a transitory increase in intracellular Ca\(^{2+}\) levels. This second-messenger signal is then mediated by Ca\(^{2+}\)-binding proteins.

A number of reports have studied the relationship between Ca\(^{2+}\) and cell functions as well as Ca\(^{2+}\) and secretion. This evidence speaks in favor of the primary regulating role of ionized Ca\(^{2+}\) in biological systems. It is also well-known that carbonic anhydrase activation is closely followed by an intracellular pH decrease, while its inhibition increases pH (Maren 1967).

Likewise, by correlating these data with findings of the present study, our results suggest that increase of carbonic anhydrase activity induced by hypercalcemia or the reduction of carbonic anhydrase activity by Ca-chelating agents might induce intracellular and extracellular pH changes. These could affect cellular
receptors, transducers and effectors involved in the physiological and pathological processes in the organism. It may thus be concluded that 1) red cell carbonic anhydrase is sensitive to changes in blood calcium levels, 2) the increase in blood calcium levels is accompanied by high carbonic anhydrase activity, while a reduction of blood calcium values reduces carbonic anhydrase activity, 3) Ca-chelating agents such as disodium EDTA reduce carbonic anhydrase activity in parallel with reduction of gastric acid secretion, and 4) the values of blood calcium can influence the activity of carbonic anhydrase, an enzyme which can modulate physiological and pathological processes in the organism by means of intracellular and extracellular pH changes.

References

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**Reprint requests**

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Calcemia mg/dl

GROUP I

GROUP II

Basal acid output (mEq/h)

GROUP I

GROUP II

* indicates statistical significance.
CA II and CA IV activity

Before treatment
After treatment

GROUP I
GROUP II

CA II
CA IV
Fig. 1

**Calcemia mg/dl**

- **GROUP I**
  - Basal: 8.5
  - After treatment: 9.3

- **GROUP II**
  - Basal: 9.84
  - After treatment: 8.58

**CA II and CA IV activity**

- **GROUP I**
  - CA II: 1.20
  - CA IV: 1.60

- **GROUP II**
  - CA II: 0.80
  - CA IV: 0.40