Influence of Calcitonin Gene-related Peptide Release on pH-induced Mechanical Depression in Rat Atria

A. Gómez ALVIS,[1] BSc, V. MILESI,[2] PhD, A. REBOLLEDO,[3] PhD, J. RAINGO,[4] BSc, *and* A. O. Grassi de GENDE,[5] MD

SUMMARY

Rat atria is richly innervated by sensory nerve fibers that release CGRP when stimulated either by capsaicin or acid pH. We studied the physiological relevance of acid pH-induced CGRP release on changes in atrial contractility and relaxation produced by lowering the pH. Isolated atria electrically paced at 2.77 Hz were exposed to a 10-minute period of metabolic acidosis (pH=6.73±0.01, n=28) after: 1) CGRP release induced by capsaicin 0.5 μM; 2) blockage of CGRP release with ruthenium red (RR) 5 μM; 3) no pretreatment; and 4) CGRP receptor blockage with $CGRP_{8-37}$ 1 μM. Contractility and relaxation were significantly less depressed by acid pH when CGRP release was prevented by RR or CGRP receptor activation was blocked by $CGRP_{8-37}$. The results suggest that CGRP release and the activation of CGRP receptors may be physiologically involved in contributing to the depression of contractility and relaxation induced by acid pH in rat atria. (Jpn Heart J 2001; 42: 507-517)

Key words: Capsaicin, Acid pH, Rat atria, CGRP receptor blockage, Ruthenium red

CAPSAICIN, the pungent active principle present in hot peppers, evokes the release of neurotransmitters from capsaicin-sensitive sensory nerve endings, ie. calcitonin gene-related peptide (CGRP), substance P (SP) and adenosine triphosphate (ATP).[1)] Acid pH is another stimulating factor for capsaicin sensitive nervous fibers[2)] and, in addition, it potentiates capsaicin-evoked current in nociceptive neurons.[3)] pH-induced release of CGRP can be abolished by the capsaicin receptor blocker capsazepine[4)] and by RR.[5)] However, it has been recently proposed that low pH could not be the endogenous activator of the cation channel stimulated by capsaicin in sensory neurons.[6)] In the isolated guinea-pig heart, capsaicin, ischemia and acid pH evoke increases in the outflow of CGRP.[7,8)] CGRP acts as a positive chronotropic and inotropic endogenous agent in both guinea-pig and rat atria.[9-11)]

From [1] Cátedra de Anatomía y Fisiología, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, [2] Postdoctoral Fellow of the Agencia Nacional de Promoción Científica y Técnica (ANPCYT). [3] Posdoctoral Fellow of the UNLP. [4] Doctoral Fellow of the UNLP. [5] Established Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) de la República Argentina.

Address for correspondence: Angela O. Grassi de Gende, MD, Casilla de Correo 219, 1900-Correo Central, La Plata-Argentina.

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There are reports describing the relevance of neurotransmitters released from capsaicin-sensitive nerves in the mechanical response of the reperfused myocardium following an ischemic period[12] as well as in the phenomenom of myocardial preconditioning.[13,14] A fall in the pH to values of 6-7 during myocardial ischemia could stimulate capsaicin-sensitive sensory fibers, so it is possible that cardiomyocytes under ischemic conditions might suffer the simultaneous effects of hypoxia, acid pH and neurotransmitters released from sensory nerves by acid pH. However, studies demonstrating an increase in CGRP outflow due to ischemia, hypoxia or acid pH have not dealt with the possible effects on myocardial contractility and relaxation of interactions between the activator factors of neurotransmitter release and the direct effects of neurotransmitters. It was of interest to us to study the contractility and relaxation in rat atria exposed to metabolic acidosis with the aim of demonstrating how acid pH-induced CGRP release and activation of CGRP receptors could modify atrial contractile responses during acidosis. We chose the isolated rat atria because this preparation is richly innervated with capsaicin-sensitive sensory nerves and also exhibits a high density of CGRP receptors.[15]

Our previous results in rat atria beating at constant frequency by electrical pacing showed that 0.5 μM of capsaicin stimulates contractility and relaxation through CGRP release because $CGRP_{8-37}$ 1 μM, an antagonist of CGRP receptors, almost completely blocked these responses to capsaicin.[16] We have also demonstrated the efficacy of 5 μM RR in preventing rat atrial capsaicin effects.[16] Thus, the effects of metabolic acidosis on contractility and relaxation were studied in different groups of atria where a) the release of neurotransmitters was evoked with 0.5 μM, capsaicin b) neurotransmitter release was blocked with 5 μM RR, and c) the CGRP receptors were blocked with 1 μM $CGRP_{8-37}$.

METHODS

Male Sprague-Dawley rats (200-250 g), cared for in accordance with international principles of animal care, were used in the present experiments. The rats were anesthetized with ethyl ether and the heart quickly removed and placed in bicarbonate-buffered salt solution equilibrated with 5% CO_2-95% O_2 at room temperature. The spontaneous beating atria were dissected and mounted in an organ bath with the free end connected to a force transducer (Letica TRI 201, Barcelona, Spain). Atria were paced with electrical field stimulation at 2.77 Hz. Isometric contractions were recorded from the amplified output of the transducer feeding an analog-digital board (DT2801, Data Translation Inc., Malboro, MA) mounted in a desktop computer. Online recordings and files were obtained with an appropriate software (Snapshot Storage Scope, EHM Data Corporation) for

late processing. High speed records (0.25 seconds/frame) were used in order to measure peak developed force (F) and, on its first derivative, maximal velocity of contraction ($+dF/dt_{max}$.) and maximal velocity of relaxation ($-dF/dt_{max}$.). The ratio $+dF/dt_{max}./-dF/dt_{max}$. was calculated to evaluate the relative effects of the interventions on velocities of contraction and relaxation, with a decrease considered to be evidence of a positive lusitropic (or relaxant) effect and an increase evidence of a negative lusitropic (or relaxant) effect.[17]

The organ bath contained bicarbonate-buffered salt solution, controlled at 37 °C, with the following composition (in mM): KCl 5, $MgSO_4$ 1, NaH_2PO_4 1, NaCl 111, $CaCl_2$ 1.35, $NaHCO_3$ 24 and glucose 11. The solution was equilibrated with 5% CO_2 (pCO_2 of 40 mmHg) and 95% O_2 (pH 7.41±0.01, n=28). Solutions with acid pH (6.73±0.01, n=28) were obtained by changing the $NaHCO_3$ concentration from 24 to 5 mM and maintaining equilibration with 5% CO_2 and 95% O_2.

After being attached to the transducer the atria were stretched until reaching maximal developed force and stabilized for 30 minutes. The bath solution was replaced every 10 minutes. After the stabilization period, records of a 10 minutes control period were obtained each minute before performing the following experimental protocols:

a. Exposure to 0.5 μM capsaicin for 10 minutes and then to acid pH for 10 minutes in the absence of capsaicin.

b. Exposure to 5 μM ruthenium red for 20 minutes and then to acid pH for 10 minutes in the presence of RR.

These protocols aimed to compare the effects of acid pH in conditions where neurotransmitter release was either stimulated (a) or prevented (b).

To explore CGRP participation in atrial mechanical changes induced by low pH, three additional protocols were performed.

c. 20 minutes at normal pH and then renewal of the solution with the same pH for 10 minutes.

d. 20 minutes at normal pH and then change to the solution with acid pH for 10 minutes.

e. 30 minutes at normal pH with addition of 1 μM $CGRP_{8-37}$ and then change to the solution with acid pH still in the presence of $CGRP_{8-37}$ for 10 minutes.

These groups allowed us to compare atrial contractile function in control (c), in metabolic acidosis being the CGRP receptors available for stimulation (d), and in metabolic acidosis being the CGRP receptors blocked (e).

Results are expressed as mean±SEM of percentage changes with respect to control values, i.e., the parameter values at the last minute before the pH change (or renewal with solution of same pH in the control group) was achieved. Statistical analysis was performed by Anova (Newman Keuls test) setting a P value of 0.05 to denote statistical significance.

Chemicals: Capsaicin (Fluka) was dissolved in methanol (Merck). Ruthenium red and $CGRP_{8-37}$ were purchased from Sigma Chemical Co. All reagents used in the preparation of the bicarbonate-buffered physiological solutions were of analytical grade.

RESULTS

The effects of metabolic pH changes from normal to acid values on atrial contractility and relaxation were first compared under two conditions: one in which sensory nerve terminals were stimulated first by capsaicin and subsequently by the change to acid pH, and the other in which sensory nerve terminal stimulation by the change to acid pH was prevented by the pretreatment, and still present after the pH change, with the cation dye RR. Capsaicin had effects *per se* on atrial contractility. Stimulation with 0.5 μM capsaicin induced maximal increases in atrial contractility ($+dF/dt_{max.}$=38±9%) and relaxation ($-dF/dt_{max.}$=41±12%) after 4 minutes (n=5). Nevertheless, there was no statistically significant difference in the control values of $+dF/dt_{max.}$ and $-dF/dt_{max.}$ the minute

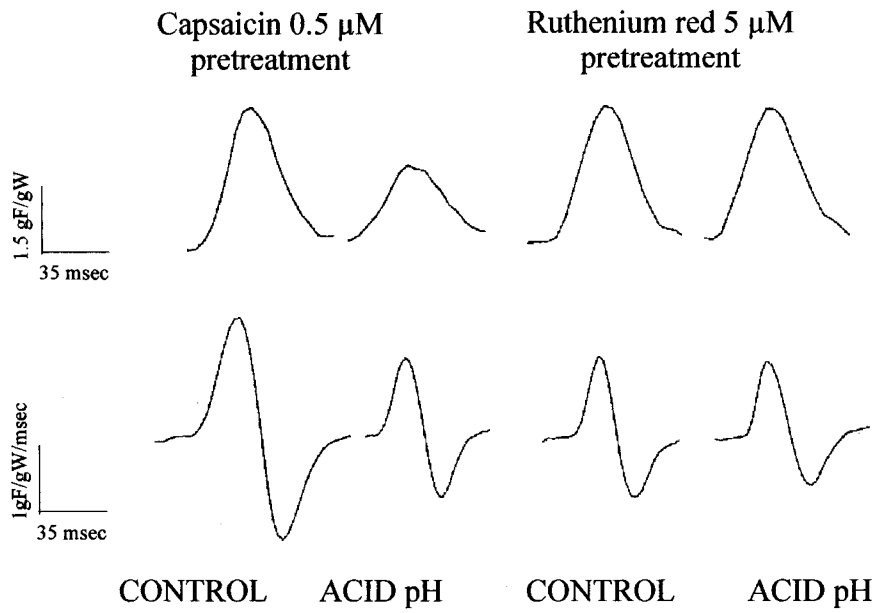

Figure 1. Typical records of force development and its first derivative from an isolated rat atrium: effects of blockade of neurotransmitter release. Left panel shows records obtained the minute preceding the pH change (control) and after the 10th minute of exposure to acid pH (acid pH) in an atrium pretreated with 0.5 μM. capsaicin Right panel shows records obtained the minute preceding the pH change (control) and after the 10th minute of exposure to acid pH (acid pH) in atrium pretreated with 5 μM RR.

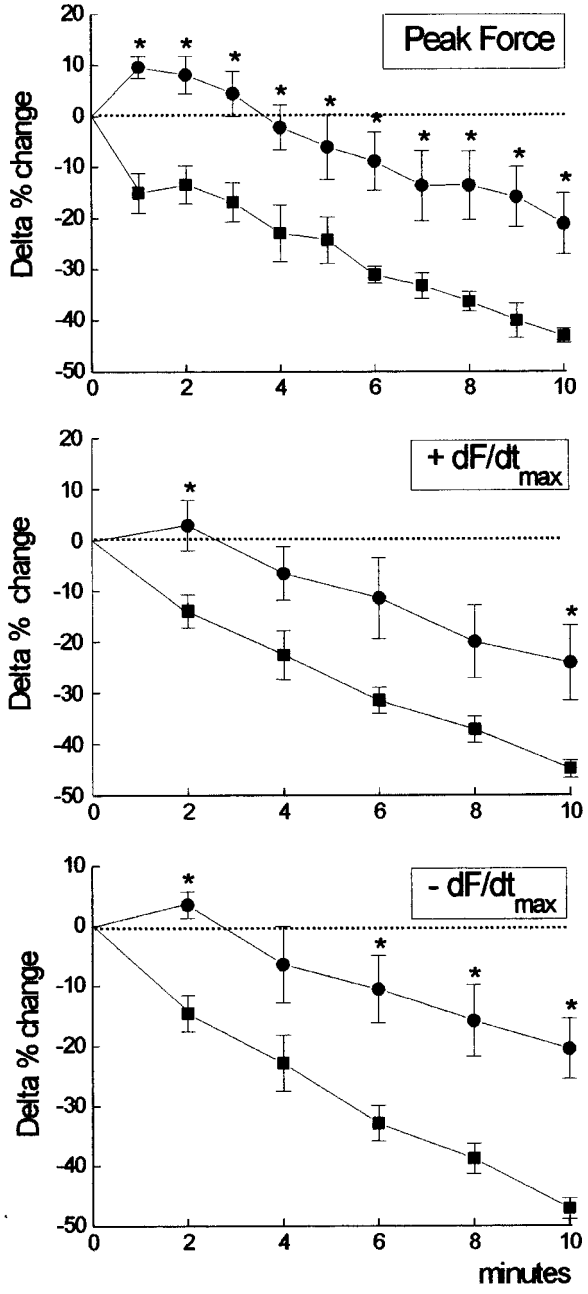

Figure 2. Mechanical changes induced by acid pH on electrically driven isolated atria: effects of blockade of neurotransmitter release. Isolated rat atria beating at 2.77 Hz were exposed to acid pH after pretreatment with 0.5 μM capsaicin for 10 minutes (■) (n=5), and after pretreatment for 20 minutes and still in the presence of 5 μM ruthenium red (RR) (●) (n=6). Results are expressed as percentage differences with respect to parameter values measured the minute preceding the change to acid pH (control values are given in the Results section). * indicates $p<0.05$ between the means of the two groups (ANOVA, Newman Keuls test).

preceding the pH change between the groups preincubated with capsaicin (0.38±0.04 and 0.34±0.03 gForce/gWeight/msec, respectively, n=5) and preincubated with RR (0.88±0.24 and 0.64±0.17 gForce/gWeight/msec, respectively, n=6). After capsaicin or RR pretreatment, the atria were exposed to acid pH. Figure 1 presents typical records obtained in these two experimental groups. The time course changes in contractility and relaxation of atria exposed to low pH are shown in Figure 2. It can be observed that the presence of RR, used to prevent neurotransmitter release from sensory nerve terminals, significantly attenuates the depression of atrial contractility and relaxation produced by low pH, with respect to the group where neurotransmitter release was stimulated by capsaicin. When the $+dF/dt_{max}$./$-dF/dt_{max}$. ratio values were compared after 10 minutes of acid pH exposure, the percentage changes indicated an improvement of relaxation in the presence of RR (4.4±1.6% vs. −3.5±2.8% in the capsaicin and RR groups, respectively; p<0.05).

Since CGRP is the neurotransmitter released by sensory nerve terminals that might have major effects on atrial mechanical function, the following protocols exposed the atria to acid pH both in the absence and presence of a CGRP receptor blocker, the peptide $CGRP_{8-37}$. There were no statis tically significant differences

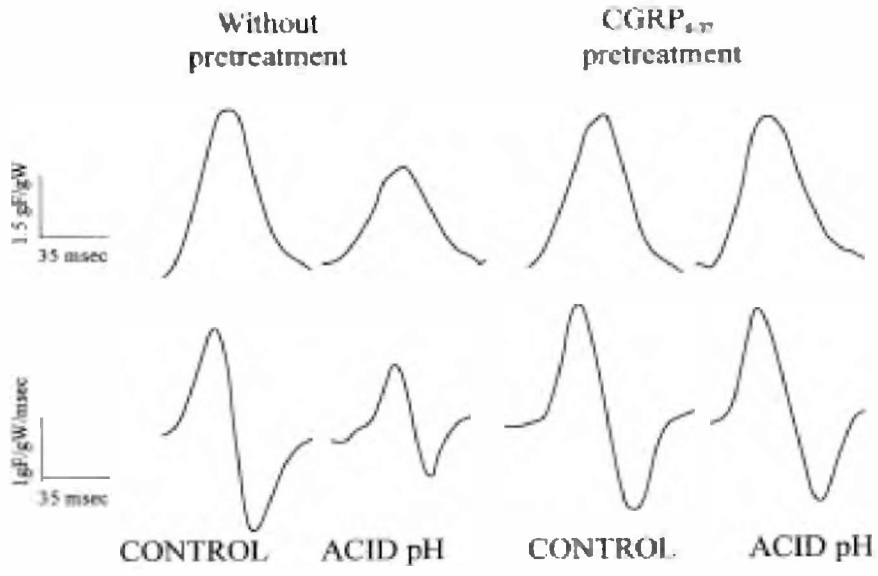

Figure 3. Typical records of force development and its first derivative from an isolated rat atrium: effects of CGRP receptor blockage. Left panel shows records obtained the minute preceding the pH change (control) and after the 10th minute of exposure to acid pH (acid pH) in atrium without pretreatment. Right panel shows records obtained the minute preceding the pH change (control) and after the 10th minute of exposure to acid pH (acid pH) in atrium pretreated with $CGRP_{8-37}$.

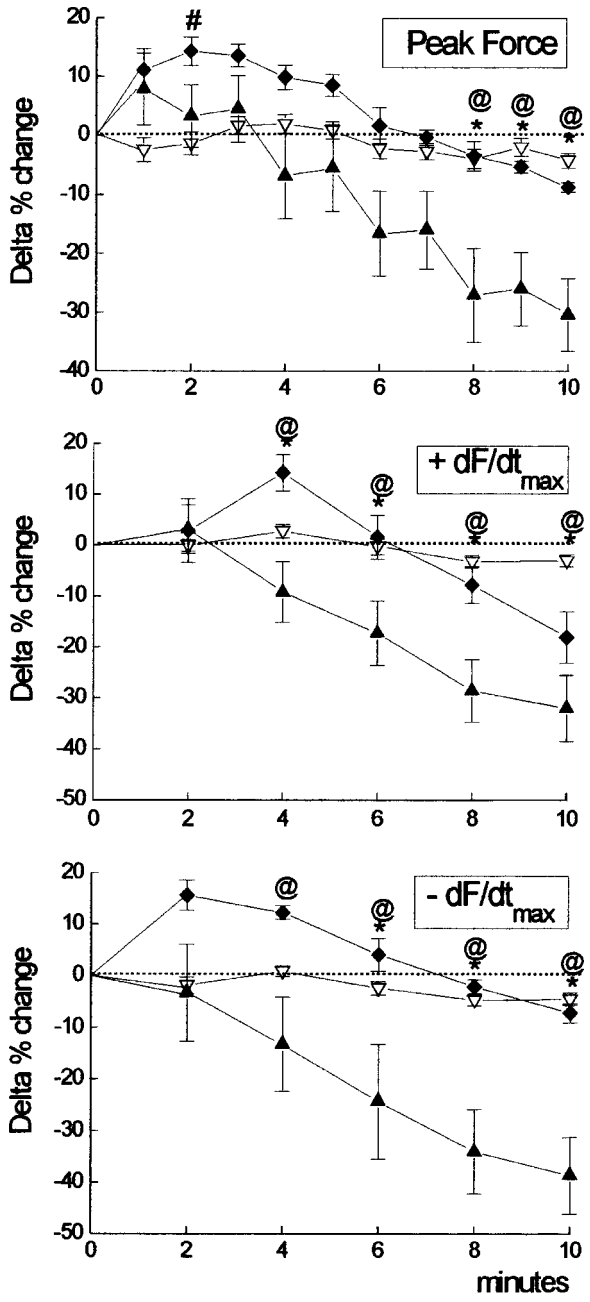

Figure 4. Mechanical changes induced by acid pH on electrically driven isolated atria: effects of CGRP receptor blockade. Isolated rat atria beating at 2.77 Hz were not exposed to acid pH (▽) (n=8), exposed to acid pH (▲) (n=6) and exposed to acid pH after pretreatment and still in the presence of the CGRP receptor blocker, $CGRP_{8-37}$ (◆) (n=4). Results are expressed as percentage differences with respect to parameter values measured the minute preceding the change to acid pH (control values are given in the Results section). The $p<0.05$ is indicated by: * control vs acid pH groups; # control vs acid pH-$GCRP_{8-37}$ groups and at acid pH vs acid pH-$CGRP_{8-37}$ groups (ANOVA, Newman Keuls test).

in the control values of $+dF/dt_{max}$. and $-dF/dt_{max}$. for the minute preceding the pH change between the groups preincubated without (0.39±0.05 and 0.40±0.09 gForce/gWeight/msec, respectively; n=6) and with $CGRP_{8-37}$ (0.51±0.04 and 0.45±0.04 gForce/gWeight/msec, respectively; n=4). Figure 3 presents typical records obtained in these two experimental groups before and after 10 minutes of exposure of the atria to acid pH. Average results comparing parameters of contractility and relaxation during the acidic period in the absence and presence of $CGRP_{8-37}$ 1 μM, against a control group without pH change (n=8), are shown in Figure 4. These results show that the blockage of CGRP receptors attenuates the acid pH-induced decreases in $+dF/dt_{max}$. and $-dF/dt_{max}$. The presence of the CGRP receptor blocker also produced a positive lusitropic effect as evidenced by the percentage fall in the ratio values ($+dF/dt_{max}./-dF/dt_{max}.$: 1.7±0.9% in the absence of $CGRP_{8-37}$ and -6±2% in the presence of $CGRP_{8-37}$ at 10 minutes of acid pH exposure; p<0.01).

DISCUSSION

This study presents evidence supporting the notion that the stimulation of neurotransmitter release from sensory nerve terminals and of CGRP receptors facilitates the deleterious effects of metabolic acid pH on rat atrial mechanical function.

Capsaicin releases peptides which are involved in the local control of cardiac chronotropism, inotropism and coronary blood flow. One of these peptides is CGRP, a neurotransmitter with capacity to increase the frequency of beating and contractility in rat atria[9-11] and to dilate coronary vessels.[18] CGRP outflow has been measured in guinea pig hearts, and it is stimulated by capsaicin and acid pH. It increased by about 1500% under a first stimulation with 1 μM capsaicin and about 150% after a second 1 μM capsaicin stimulation; under acid pH values of 6-7 CGRP outflow increased 250%.[5] On the basis of these figures, our experimental conditions, treatment of rat atria with 0.5 μM capsaicin and/or pH of about 6.7, should be appropriate stimuli for CGRP release from sensory nerve terminals. We have previously reported that 0.5 μM capsaicin increased contractility and relaxation in electrically paced rat atria and that 1 μM $CGRP_{8-37}$ effectively prevented these capsaicin effects.[16]

In electrically paced atria acid pH produced a deterioration in contractility in the group pretreated with capsaicin. We originally thought that acid pH would still liberate CGRP after capsaicin treatment and that the presence of this peptide, due to its own positive inotropic effects, will attenuate the depression of contractility and relaxation induced by acidosis. However, the blockage of neurotransmitter release with RR showed that contractility and relaxation were less

depressed by the change to acid pH compared to atria in which neurotransmitter release was allowed. In spontaneously beating atria we have previously observed that acid pH in the presence of RR enhanced contractility, while after capsaicin pretreatment acid pH depressed it (data not shown). The present results at a constant atrial frequency of beating raised again the possibility that the CGRP released from sensory nerve terminals cooperated to produce depression of atrial contractility and relaxation during acidosis. This possibility was investigated by blocking the CGRP receptors by exposing the atria to $CGRP_{8-37}$ prior to and during the acidic period. In agreement with the results showing an attenuation of the negative inotropic effects of acid pH by prevention of neurotransmitter release with RR, we found that when CGRP receptor activation was blunted atrial contractility and relaxation were significantly less depressed by the acid pH. Particularly, atrial relaxation during the acidic period was more improved than atrial contractility in both groups of atria lacking CGRP receptor activation.

In Langendorff perfused rat hearts paced at 3 Hz, metabolic acidosis of pH 6.8 elicited a negative inotropic effect which was quantitatively similar to the one observed in our experimental conditions in rat atria.[19] Extracellular acid pH affects myocardial function by lowering intracellular pH, which in turn may modify calcium fluxes through both plasmalemmal[20,21] and sarcoplasmic reticulum[22-24] membranes as well as myofilament sensitivity to Ca^{2+}. Because hyperbaric acidosis produces increases in diastolic cytosolic and mitochondrial calcium levels in isolated rat cardiomyocytes that course with a decrease in cell shortening,[25] it is possible to propose that the major effect of intracellular acidosis mediating a decrease in cardiac contractility is the decrease in myofilament calcium sensitivity.[22,26,27] Several opposite effects caused by CGRP and acid pH have been reported in the literature, i.e. CGRP prolongs action potential duration[28] while acid pH shortens it,[29] and CGRP promotes Ca^{2+} entry through voltage activated Ca^{2+} channels[30] while acid pH inhibits it.[20]

Our results suggest that CGRP would be cooperating with acid pH in decreasing rat atrial contractility and relaxation. CGRP activates adenylate cyclase and elevates cAMP intracellular levels.[31] Protein kinase A-dependent phosphorylation of cardiac troponin decreases its affinity for calcium[32] and therefore this cAMP-dependent effect would be synergistic with the lower calcium sensitivity of cardiac myofilaments induced by acid pH. Other synergistic effects of CGRP and acid pH would be the activation of potassium channels whose current is dependent on intracellular ATP concentration because both CGRP and acid pH increase the K^+ current carried through these channels in cardiac myocytes.[33,34] These two coincident effects of CGRP and acid pH can negatively influence inotropism in rat atria. The fall in the $+dF/dt_{max}./-dF/dt_{max}$. ratio values demon strates that both blockage of CGRP release and CGRP receptors improves

relaxation when the atria were exposed to low pH.

In summary, the neurotransmitters released by capsaicin and/or acid pH and the stimulation of CGRP receptors contribute to the depression of contractility and relaxation elicited by metabolic acidosis in rat atria. The results suggest a physiological involvement of CGRP release and activation of CGRP receptors in the myocardial response to acidosis, hypoxia and ischemia.

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