Modulation of Coronary Flow and Cardiomyocyte Size by Sensory Fibers

Angelina Zanesco, Soraia K.P. Costa, Sonia R. Riado, Luciana P. Nathan, Claudia F. de Oliveira, Iara M.S. De Luca, Edson Antunes, Gilberto De Nucci

Abstract—Cardiac tissue is densely innervated by sensory neurons that are believed to play important modulatory roles in cardiac functions. In this study, pretreatment of neonate rats with capsaicin was performed. In adult rats, cardiomyocyte size and amount of fibrous tissue in left ventricles as well as in vitro coronary flow were evaluated. The chronotropic and inotropic responses to β-adrenoceptor agonists (norepinephrine and isoproterenol), muscarinic agonists (carbachol and pilocarpine), and calcitonin gene–related peptide (CGRP) were also investigated with the use of the isolated right atria preparation. Capsaicin pretreatment significantly (P<0.05) reduced both basal coronary flow (18% reduction) and cardiomyocyte size (34% reduction) without affecting the amount of fibrous tissues in the left ventricles. The positive inotropic and chronotropic effects in response to norepinephrine in the isolated rat heart did not significantly differ between control and capsaicin-treated rats. Similarly, the positive chronotropic effects in response to norepinephrine, isoproterenol, and CGRP as well as the negative chronotropic responses to carbachol and pilocarpine in the isolated right atria were not affected by capsaicin pretreatment. Our data are consistent with the suggestion that reductions of both basal coronary flow and cardiomyocyte size seen in hearts from capsaicin-pretreated rats may be consequences of CGRP depletion. The cardiomyocyte size reduction produced by capsaicin treatment may be related to a modulatory role of CGRP as a growth factor. (Hypertension. 1999;34[part 2]:790-794.)

Key Words: capsaicin receptors, muscarinic receptors, adrenergic, beta receptors, neuropeptides peptides

Sensory fibers have been associated with the control of smooth muscle tone, autonomic ganglia transmission, immunologic processes, tissue growth,1 and heart functions.2–5 A number of substances, including the peptides substance P and calcitonin gene–related peptide (CGRP), are released by the sensory nerve endings.1,6 Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a highly selective neurotoxin that, on systemic administration, causes CGRP and substance P depletion from sensory neurons7–9 and thus has largely been used to study the involvement of sensory fibers in different pathophysiological functions.10

In the heart, capsaicin increases contractile force and spontaneous heart rate3 as well as evokes coronary vasodilation through CGRP release.11,12 Previous studies reported the presence of CGRP in the heart, predominantly in the right atria, followed by the left atria and right and left ventricles,3,13 where it causes concentration-dependent and long-lasting positive inotropic and chronotropic effects in several species,9,14–17 including humans.18 Although CGRP has also been described as a potent hypertrophic factor for cardiomyocytes,19 no study has been performed to investigate the effect of CGRP depletion on cardiomyocyte size. The existence of interactions of sympathetic and parasympathetic nerves with sensory fibers in vitro20,21 and in vivo22 preparations has been reported.23 In this study, pretreatment of neonate rats with capsaicin was performed and the rats were used at adult ages. We then evaluated the following parameters in the heart: (1) cardiomyocyte size, (2) basal and stimulated coronary flow in vitro, and (3) chronotropic and inotropic responses of β-adrenoceptors and muscarinic agonists as well as CGRP, with both whole rat isolated heart and isolated right atria.

Methods

Capsaicin Treatment

The experiments were performed in Wistar rats of both sexes bred in the department of Animal Care of the Faculty of Medical Sciences, State University of Campinas (São Paulo, Brazil). In total, 102 neonatal rats were pretreated subcutaneously on the second day of life with capsaicin (50 mg/kg; Sigma Chemical Co) or the corresponding volume (100 μL) of capsaicin-vehicle (10% ethanol and 10% Tween 80, in 0.9% [wt/vol] NaCl solution) under ether anesthesia.8 Rats were used 12 to 14 weeks after capsaicin pretreatment, at which time they weighed 200 to 300 g. All procedures were designed in accordance with the guidelines of the State University of Campinas for animal care.

Received May 8, 1999; first decision June 22, 1999; revision accepted July 10, 1999.

From the Department of Physical Education, Biosciences Institute (A.Z.), Paulista State University, Rio Claro (SP) and the Department of Pharmacology, Faculty of Medical Sciences (S.K.P.C., S.R.R., L.P.N., C.F. de O., E.A., G. De N.); and Department of Histology and Embryology, Biology Institute, State University of Campinas, (I.M.S. De L.), Campinas (SP), Brazil. Correspondence to Angelina Zanesco, PhD, Department of Physical Education, Biosciences Institute, Paulista State University, Av 24A No. 1515, Bela Vista, CEP 13506-900, Rio Claro (SP), Brazil. E-mail azanesco@bestway.com.br © 1999 American Heart Association, Inc.

Hypertension is available at http://www.hypertensionaha.org
Langendorff Preparation

Male Wistar rats (weight, 250 to 350 g) were anesthetized with sodium pentobarbital (Sagatal; 50 mg/kg IP) and given heparin (500 IU/kg IP) 5 minutes before thoracotomy. The hearts were rapidly excised and mounted on a Langendorff apparatus and perfused at constant pressure (65 mm Hg) with oxygenated (95% O₂/5% CO₂) Krebs-Henseleit solution containing (in mmol/L) NaCl 118, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25, and glucose 11, pH 7.4, at 37°C. Left ventricular developed pressure (LVPD) (mm Hg) and heart rate (HR) (bpm) were recorded via a latex balloon inserted into the left ventricle (basal end-diastolic pressure, ~5 mm Hg). A polyethylene cannula was then connected to a pressure transducer (model PRC 213, Ugo Basile) and a 2-channel recorder (Gehani 7070, Ugo Basile). The preparations were allowed to stabilize for 30 minutes. Coronary flow (ml/min) was measured manually by 15-second timed collections of the coronary effluent.

Functional Assays With Isolated Right Atria

Animals were anesthetized with halothane, and the hearts were rapidly removed. The right atria were isolated and mounted in a water-jacketed tissue chamber (10-mL volume) containing Krebs-Henseleit solution, pH 7.3 to 7.5, at 37°C and gassed with 95% O₂/5% CO₂. The composition of the Krebs-Henseleit solution was as follows (mmol/L): NaCl 124, KCl 4.75, MgSO₄ 1.30, CaCl₂ 2.25, NaHCO₃ 25, and glucose 11, pH 7.4, at 37°C. Left ventricular developed pressure (LVPD) (mm Hg) and heart rate (HR) (bpm) were recorded via a latex balloon inserted into the left ventricle (basal end-diastolic pressure, ~5 mm Hg). A polyethylene cannula was then connected to a pressure transducer (model PRC 213, Ugo Basile) and a 2-channel recorder (Gehani 7070, Ugo Basile). The preparations were allowed to stabilize for 30 minutes. Coronary flow (ml/min) was measured manually by 15-second timed collections of the coronary effluent.

Concentration-Response Curves

Concentration-response curves for the positive and negative chronotropic actions of isoproterenol, norepinephrine, rat CGRP, carbachol, and pilocarpine (all Sigma Chemical Co) were constructed by the cumulative variation of agonist concentration at one-half log unit increments. Additionally, stereological analysis of the left ventricle revealed a 34% reduction (P<0.05) of cardiomyocyte size in the capsaicin-pretreated rats without change in the amount of fibrous tissues (Table 1).

Stereological Procedures

Stereological analysis was performed according to the method described by Aherne.₂₅ Formalin-fixed left ventricle and septum were cut into 5 equidistant rings perpendicular to the long axis of the ventricle. The rings were then embedded in paraffin, and 5-μm sections were stained with Masson’s trichrome. Analysis of the slides was performed in blinded fashion on a light microscope (Zeiss), and the relative volume occupied by each element of the ventricle (myocardial fibers and fibrous tissue) was measured with a special ocular apparatus containing a 25-point reticulum (5 parallel lines with 5 points each, kpl × 8, Zeiss). To determine cardiomyocyte size, 15 cells randomly selected from the subepicardial, midmyocardial, and subendocardial regions were measured for each animal from the different experimental groups. For counting, 50 microscopic fields were evaluated, and the relative volume (Ppi) occupied by each element was calculated as follows: Ppi =

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Capsaicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>116±2</td>
<td>113±2.2</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>434±43</td>
<td>400±16</td>
</tr>
<tr>
<td>Myocyte size, μm</td>
<td>17.8±0.25</td>
<td>11.8±0.5*</td>
</tr>
<tr>
<td>Fibrous tissue, %</td>
<td>5.8±0.4</td>
<td>5.8±0.09</td>
</tr>
</tbody>
</table>

Values are mean±SEM of 5 (control) to 12 (capsaicin) experiments. *P<0.05 vs control group.

Blood Pressure Measurement

The systolic blood pressure was measured by a modified tail-cuff method in awake animals. The measurements were performed 24 hours before the animals were killed.

Statistical Analysis

All values are expressed as mean±SEM. The program InStat (GraphPad Software) was used for statistical analyses. When appropriate, 1-way ANOVA followed by a Bonferroni multiple comparisons post hoc test was performed to determine whether the treatments had an effect. In some cases, a paired or unpaired Student’s t test was used. P<0.05 was accepted as significant.

Results

Body Weight, Stereological Analysis, and Systolic Blood Pressure

Body weight and systolic blood pressure were not significantly affected by the capsaicin pretreatment (n=12) compared with the control group (n=5; Table 1). Additionally, stereological analysis of the left ventricle revealed a 34% reduction (P<0.05) of cardiomyocyte size in the capsaicin-pretreated rats without change in the amount of fibrous tissues (Table 1).

Whole Heart Preparations

Bolus injection of norepinephrine (0.6 nmol) caused similar increases in LVDP and HR in both control and capsaicin-treated animals (Table 2). Capsaicin pretreatment significantly reduced basal coronary flow by ~18% (P<0.05; Table 2). Bolus injection of norepinephrine (1.0 nmol) caused a significant decrease in coronary flow in the control group at 0.25 minutes after injection (35% reduction), whereas in capsaicin-treated rats the reduction in coronary flow by norepinephrine was attenuated (18.7% reduction; Table 2).

Isolated Right Atria

Addition of capsaicin (1 μmol/L) to the organ bath caused a positive chronotropic response in isolated right atria of the control group (44±4 bpm) that was attenuated by ~45% (P<0.05) in capsaicin-treated rats (20±2 bpm). Chronotropic responses to both β-adrenoceptor (isoproterenol and norepinephrine) and muscarinic (carbachol and pilocarpine) agonists are illustrated in the Figure. There was no significant shift of the concentration-response curves for
norepinephrine between control and capsaicin groups (Figure, panel A). With respect to isoproterenol, although there was a tendency for a rightward shift in the concentration-response curves, the differences between both groups were not significant (Figure, panel B). The potency of muscarinic agonists was also unaffected by capsaicin pretreatment in isolated right atria (Figure, panels C and D).

Rat CGRP induced concentration-dependent positive chronotropic effects of rat right atria that were ~30% of maximum response for isoproterenol and norepinephrine, as observed in either control or capsaicin groups (Table 3). Furthermore, there were no significant changes in the potency of rat CGRP in isolated right atria after capsaicin pretreatment compared with the control group (Figure, panel E). The maximum responses for all studied agonists were similar in capsaicin-treated rats and the control group (Table 3). Table 4 summarizes all studied agonists in isolated right atria from control and capsaicin-treated rats.

**TABLE 2. Effect of Norepinephrine Bolus Injection (0.6 nmol) on LVDP, HR, and Coronary Flow in Whole Heart From Control and Capsaicin-Pretreated Rats**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>LVDP, mm Hg</th>
<th>HR, bpm</th>
<th>Coronary Flow, mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>98±9.5</td>
<td>319±16</td>
<td>13.3±1.8</td>
</tr>
<tr>
<td>0.25</td>
<td>131±15*</td>
<td>348±20*</td>
<td>8.9±1.1*</td>
</tr>
<tr>
<td>1</td>
<td>106±11</td>
<td>315±23</td>
<td>12.5±0.2</td>
</tr>
<tr>
<td>5</td>
<td>99±10</td>
<td>337±15</td>
<td>11.9±0.5</td>
</tr>
<tr>
<td>10</td>
<td>99±9.0</td>
<td>330±16</td>
<td>11.7±0.7</td>
</tr>
</tbody>
</table>

Data are mean±SEM for 5–20 experiments.

*P<0.05 compared with respective basal values.

†P<0.05 compared with control group.

Concentration-response curves to norepinephrine (A), isoproterenol (B), carbachol (C), pilocarpine (D), and rat CGRP (E) in isolated right atria from control (○) and capsaicin-treated (●) rats. Values are mean±SEM of 5 to 8 experiments.
CGRP has been described as a growth factor in the rat heart, where it exerts beneficial trophic actions on cardiomyocytes.1 Accordingly, our stereological studies have evidenced a decrease in cardiac muscle fiber size in capsaicin-treated rats. These results are therefore consistent with the suggestion that CGRP plays an important role in the maintenance of cardiomyocyte size under normal conditions by acting as a growth factor. Additionally, constant coronary flow as a consequence of continuous release of CGRP may also contribute to regulation of cardiac muscle fiber size. Reduction in coronary flow leads to an increase in the fibrous tissue in the heart as a result of ischemic processes.36 However, capsaicin-treated rats showed no alterations in the amount of fibrous tissues in the left ventricles, suggesting that the magnitude of the coronary flow reduction was insufficient to determine ischemic processes.

The positive chronotropic responses for isoproterenol, norepinephrine, and rat CGRP and the negative chronotropic responses for carbachol and pilocarpine were not affected in isolated right atria by capsaicin treatment. These results may be explained by the partial depletion (45%) of neuropeptides in our study. However, this is unlikely since previous work also found no alterations in the potency of both agonists, isoproterenol and rat CGRP, in right atria from guinea pigs and rats treated with capsaicin, where complete depletion of CGRP was detected.37 In a manner similar to that of chronotropism, capsaicin pretreatment had no effect on the inotropic responses to norepinephrine in isolated whole heart, indicating that cardiomyocytes may be able to retain their physiological responses, including contraction force, despite their size reduction. Taken together, these observations presumably indicate that sensory fibers do not play a role in the primary modulation of cardiac actions (chronotropism and inotropism) compared with the sympathetic nervous system, in which norepinephrine is the main neurotransmitter.

In summary, capsaicin treatment in neonatal rats causes a significant decrease of basal coronary flow, associated with diminishing cardiomyocyte size, as a consequence of CGRP depletion.

### References


Modulation of Coronary Flow and Cardiomyocyte Size by Sensory Fibers
Angelina Zanesco, Soraia K. P. Costa, Sonia R. Riado, Luciana P. Nathan, Claudia F. de Oliveira, Iara M. S. De Luca, Edson Antunes and Gilberto De Nucci

Hypertension. 1999;34:790-794
doi: 10.1161/01.HYP.34.4.790

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/34/4/790

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/