Effects of K⁺ Channel Modulators on Oscillatory Contractions in Sinoaortic Denervated Rat Aortas

Matheus Lavorenti Rocha¹ and Lusiane Maria Bendhack*²,

¹ Department of Pharmacology, Faculty of Medicine of Ribeirão Preto University of Sao Paulo; Av. Bandeirantes, 3900, 14049–900, Ribeirão Preto, SP, Brazil; and ² Department of Physics and Chemistry, Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of Sao Paulo; Av. do Café s/n, 14040–903, Ribeirão Preto, SP, Brazil. Received June 30, 2007; accepted August 21, 2007

Sinoaortic denervated (SAD) rats present arterial pressure lability without sustained hypertension. We investigated the relation between sinoaortic denervation and the occurrence of oscillatory contractions in SAD rat aortas, as well as the effect of various K⁺ channel modulators on these oscillations. Aortas were removed and concentration–effect curves to phenylephrine (0.01 to 10 μg) were constructed in arteries from SAD and Sham-operated rats in order to verify the occurrence of oscillations. We also evaluated the effects of various K⁺ channel modulators on these oscillations. Only SAD rat aortas exhibited oscillatory contractions. Tetraethylammonium increased the frequency (28.5±3.5 to 41.5±4.5 counts/5 min) and amplitude (0.435±0.07 to 0.630±0.09 g) of the oscillations. Apamin and 4-aminopyridine did not alter the oscillations. Barium chloride converted the oscillatory contractions to a tonic contraction. Pinacidil rapidly blocked the oscillatory contractions and glibenclamide evoked reduction in amplitude from 0.410±0.07 to 0.180±0.06 g. Iberiotoxin increased the frequency of oscillatory contractions (from 28.0±3.5 to 51.5±7.5 counts/5 min) but decreased the amplitude (from 0.410±0.08 to 0.195±0.2 g). Our results demonstrate that SAD rat aortas exhibit oscillatory contractions and K⁺ channels, mainly KATP and BKCa, play a dominant role in these oscillations.

Key words oscillatory contraction; sinoaortic denervation; potassium channel; rat aorta

Sinoaortic denervation (SAD) disrupts the arterial baroreceptor reflex system that mediates the regulation of blood pressure and heart rate.¹,² Immediately after SAD there is an increase in sympathetic activity that is characterized by a labile hypertension and tachycardia. There is general agreement that SAD causes a substantial increase in arterial pressure lability without changes in the mean level of blood pressure.³–⁶ Therefore, SAD rats can be used to study physiological changes caused by high arterial pressure lability without sustained hypertension.

Many previous studies have shown that some isolated smooth muscle preparations generate spontaneous oscillatory contractions, and this oscillatory behavior could also be stimulated by chemical stimuli.⁷–¹⁰ The effect of oscillatory contractions on vascular beds is currently controversial, so its physiological significance is yet to be correctly defined.

Despite the many studies exploring oscillatory contractions in the smooth muscle, the fundamental reasons underlying these events still remain unclear. One of the reasons for the lack of understanding of the oscillatory behavior is the fact that probably more than one mechanism causes oscillatory contractions and these mechanisms must be complex. Therefore, there is an urgent need for experimental approaches that can help understand the mechanisms that evoke oscillatory contractions. Nevertheless, there are few studies exploring these oscillatory events in large arteries, such as the aorta.

In an attempt to explore the mechanism involved in the maintenance of the oscillatory contractions in smooth muscle preparations, numerous studies have demonstrated the importance of the different types of K⁺ channels for the oscillations. Furthermore, the participation of the different types of K⁺ channels can vary depending on the initial oscillatory incentive and on the vascular beds being studied. For example, Ca²⁺-activated K⁺ channels (KₐCa) play an important role in the maintenance of the oscillations and their blockade inhibits oscillatory contractions in the rat basilar artery,¹¹ rat mesenteric artery,¹,¹² and rabbit ear artery.¹³ In contrast, the KᵥCa blockades have no effects on the oscillations in the rat tail arteries.¹⁴ While it is responsible for amplifying the oscillations in the guinea-pig urinary bladder.¹⁵ Moreover, the KᵥCa blockade is responsible for starting and maintaining the oscillations in the guinea-pig trachea.⁹⁰

On the basis of this contradictory information, the aim of the present study was to investigate the relationship between the oscillatory contractions of SAD rat aortas, recently described¹⁵ and K⁺ channels. The effect of various K⁺ channel modulators on the oscillatory contractions in the SAD rat aortas were probed in the present study using several relatively specific modulators, which include the K⁺ channel blockers tetraethylammonium (TEA), 4-aminopyridine (4-AP), barium chloride, glibenclamide, apamin, iberiotoxin as well as the K⁺ channel activator pinacidil.

MATERIALS AND METHODS

Sinoaortic Denervation All the procedures were carried out in accordance with the Ethical Animal Committee, of the University of São Paulo, Brazil. Male Wistar rats (180 to 210 g) were used throughout the study and divided into two groups: sinoaortic denervated rats (SAD) and sham-operated rats (SO). Bilateral surgeries were performed according to the method reported by Krieger (1964).¹¹ Briefly, the rats were anesthetized with ketamine (50 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.), and suitably fixed in a supine position. An extensive (3–4 cm) middle incision was made into the neck, and the bilateral sternohyoid muscle was resected, exposing the neurovascular sheath. The common carotid arteries and the vagal trunk were isolated carefully. The aortic depressor nerves (except those traveling with the recurrent la-
ryngeal and superior laryngeal nerves) were thus severed. The fibers of the latter type were also interrupted by resection of the superior laryngeal nerves. The neck muscles were separated carefully, to widely expose the carotid bifurcation. The bifurcation and all the carotid branches were stripped of fibers and connective tissue. The sham surgery consisted of the same procedure, but no denervation was carried out. After the SAD or the SO surgery, polyethylene catheter consisting of a piece of heat-stretched PE10 tubing fused to a PE50 extension containing heparin at 100 IU/ml in 0.9% saline solution were implanted in the left femoral vein and artery for the subsequent administration of phenylephrine, as well as recording of the arterial pressure and heart rate. Catheter was tunneled subcutaneously and exteriorized at the dorsal neck region. Following surgery, all animals were treated with oxytetracycline (200 mg/kg, i.m.), to minimize infection. The rats were housed in individual cages with controlled temperature (22±1 °C), under a 12 h light–dark cycle and with free access to standardized rodent chow and tap water. Three days after the surgery, the arterial pressure and heart rate were measured by connecting the arterial catheter to a pre-calibrated pressure transducer coupled with the amplifier recorder, and the responses were recorded using a computerized system and a Chart software 4.0 (PowerLab, ADInstruments). The total sinoaortic denervation was evaluated by determining the change in heart rate response to a 40±10 mmHg increase in arterial pressure produced by the intravenous injection of phenylephrine 3–4 µg/kg. Only rats that exhibited bradycardia of less than 20 beats/min were considered to be sinoaortic denervated rats.

Arterial Pressure Recordings Three days after surgeries (SAD and SO), the arterial pressure was recorded in conscious rats for at least 30 min. The arterial pressure and heart rate signals were digitalized by a microcomputer and the values were determined and averaged for 30 min of uninterrupted recordings. A widely used method for determining arterial pressure lability is the calculation of the standard deviation of the arterial pressure recorded continuously for a certain range of time. 15–18) Arterial pressure lability was expressed by the standard deviation of the mean value of the arterial pressure.

Preparation of Rat Aorta Three days after the surgeries, rats were killed by decapitation and the thoracic aorta was quickly removed, dissected free, and cut into 4-mm-long rings. The aortic rings were placed between two stainless steel stirrups and connected to an isometric force transducer (Leticia Scientific Instruments) coupled to a PowerLab data acquisition unit. The responses were recorded using a computerized system and a Chart software 4.0 (PowerLab, ADInstruments), to measure tension in the vessels. The rings were placed in a 10 ml organ chamber containing modified Krebs solution with the following composition (mM): NaCl 130, KCl 4.7, KH2PO4 1.2, MgSO4 1.2, NaHCO3 14.9, glucose 5.5, CaCl2 1.6. The solution was maintained at pH 7.4, and gassed with 95% O2 and 5% CO2 at 37 °C. The rings were initially stretched to a basal tension of 1 g (previously determined by length–tension relationship experiments), before allowing them to equilibrate in the bathing medium. The endothelium was mechanically removed by gently rolling the lumen of the vessel on a thin wire, and its integrity was qualitatively assessed by the degree of relaxation caused by 1 µM acetylcholine in the presence of contractile tone induced by 0.1 µM phenylephrine. The rings were discarded when there was some degree of relaxation to avoid the possible influence of endothelial factors.

Data Collection and Measurement of Oscillatory Contractions Oscillatory contractions were induced by low cumulative aliquots of phenylephrine (5 to 15 nM) until the beginning of oscillations. After that, they stayed stable for up to 1 h, without alteration in frequency and amplitude. Before applying test compounds (K+ channel modulators), oscillatory contractions force was observed for 15 min after reaching the relatively steady-state level. Subsequently, test compounds were applied to the bath medium, and the changes in the oscillatory contractions were observed for 20 min. The responses of the last 5 min period during 20 min time observation were used for analysis.

Oscillatory contractions were graphically expressed as average contraction amplitude and its frequency during 5 min. First of all, the basal tension (bottom of oscillatory wave) of the aortic rings was defined as the exhibited tension for the last 5 min before and after the drugs addition. These definitions are shown in Fig. 2. In order to evaluate the effect of the compounds on the oscillatory contractions, the tension, contraction amplitude and frequency, were expressed in comparison to the control values obtained in the absence of the K+ channel modulators.

Drugs The drugs used in this study were: Phenylephrine hydrochloride, acetylcholine hydrochloride, tetraethylammonium (TEA), glibenclamide, 4-aminopyridine (4-AP), Iberotoxin (IbTX), and apamin, purchased from Sigma Chemical Co., (St. Louis, MO, U.S.A.). Pinacidil was purchased from Research Biochemicals International (South Natick, MA, U.S.A.). All other chemicals used in the present study were of reagent grade. Glibenclamide, 4-aminopyridine and pinacidil were dissolved in DMSO (0.1% of total volume) and diluted with distilled water. The other drugs were dissolved in distilled water. The concentration of DMSO in the organ chamber did not exceed 0.01% and had no effect on tension generation in vascular rings in this preparation.

Statistical Analysis All data of oscillatory contractions are presented as means±S.E.M. In each set of experiments, n indicates the number of rats studied. The statistical analysis was performed using the GraphPad Prism version 3.0 (GraphPad Software Corporation, San Diego, CA, U.S.A.). Comparisons among groups were performed using ANOVA (post-test Newman–Keuls) and Student’s t test, and values of p<0.05 were considered to be significant.

RESULTS Arterial Pressure Variability and Emergence of Oscillatory Contractions Figure 1a shows the mean arterial pressure recordings of rats 3 d after SAD and SO. As shown in the upper tracings, the arterial pressure lability was observed in SAD rats only. On the other hand, the mean arterial pressure was not raised in SAD rats (114±6.2 mmHg, n=12) compared to SO rats (107±5.4 mmHg, n=8). However, the arterial pressure lability (standard deviation of the mean arterial pressure) was obviously increased in the case of the SAD rats (15.3±3.1 mmHg) as compared to the SO rats (3.6±1.7 mmHg). The values of systolic and diastolic blood
pressure in SAD rats (125±8 mmHg and 97±10 mmHg, respectively) were not different as compared to SO rats (123±7 mmHg and 95±11 mmHg, respectively). In isolated aortic rings of both groups (SAD and SO rats), increasing concentrations of phenylephrine induced contractile responses in both aorta groups, but we observed the emergence of oscillatory contractions in SAD rat aortas only (Fig. 1b). These oscillations in the vascular tension occurred at an intermediate phenylephrine concentration (10 to 50 nM). These oscillations were observed in all aortic rings isolated from SAD rats.

Characterization and Analysis of Oscillatory Contractions in SAD Rats Aorta
In this work, we attempted to verify the role of different K\(^+\) channels in the oscillatory contractions that are myogenic in origin. For this reason, the oscillatory contractions were analyzed in denuded isolated aortas in order to avoid the endothelium influence on the responses. Under phenylephrine stimulus, the frequency of oscillatory contractions was 27.5±3 counts for 5 min, with an amplitude of 0.415±0.08 g, and the basal tension was 0.305±0.03 g (n=10 for each). All these parameters (frequency, amplitude and basal tension, see Fig. 2) were measured before and after addition of the different K\(^+\) channel modulators.

**Effects of Non-selective K\(^+\) Channel Blockers on the Oscillatory Contractions**
To investigate the role of K\(^+\) channels on the oscillatory contractions, we analyzed the effect of the non-selective K\(^+\) channel blocker tetraethylammonium (TEA, 5 mM). TEA increased the tension from 0.312±0.03 to 1.250±0.25 g (Fig. 3b). Moreover, TEA increased the frequency from 28.5±3.5 to 41.5±4.5 counts for 5 min. The amplitude was also increased from 0.435±0.07 to 0.630±0.09 g (n=7). As shown in Fig. 3, TEA increased the tension (b), frequency (c) and amplitude (d) of the oscillatory contractions as compared to control values. The numerical values are also shown in Table 1. In an attempt to verify the role of L-type Ca\(^{2+}\) channels, we investigated the effect of the Ca\(^{2+}\) channel blocker verapamil on the oscillatory contractions after addition of TEA. The lower verapamil concentration tested in this work (0.3 µM) interrupted the oscillatory contractions, although it did not reduce the tension. The elevation in the basal tone evoked by TEA was inhibited by 1 µM verapamil, so the tension returned to baseline (Fig. 3).

**Effect of a Voltage-Gated K\(^+\) Channel (K\(_v\)) Blocker on Oscillatory Contractions**
In a separate series of experiments, oscillatory contractions were expressed in the graphics as the amplitude and frequency of tension changes during a 5 min period. Oscillatory contractions occurred spontaneously or they were induced by very low concentrations of phenylephrine (shown as arrows) until the beginning of the oscillations. After that, the oscillatory contractions were maintained stable for up to 1 h, without changes in the frequency or amplitude.
Oscillatory Contractions in the SAD Rat Aortic Rings

Contractions. After the addition of barium (30 \mu M), there was a significant increase in tension from 0.305 \pm 0.03 to 0.515 \pm 0.07 g (n=5) as shown in Fig. 4b, but did not modify either the frequency (Fig. 4c) or the amplitude (Fig. 4d) of the oscillations. The absolute values are presented in Table 1.

<table>
<thead>
<tr>
<th>Channel Modulators</th>
<th>Tension (g)</th>
<th>Frequency (counts/5 min)</th>
<th>Amplitude (g)</th>
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<tr>
<td>TEA (5 \mu M)</td>
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<tr>
<td>Control</td>
<td>0.312\pm0.03</td>
<td>28.5\pm3.5</td>
<td>0.435\pm0.07</td>
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<tr>
<td>Treated</td>
<td>1.250\pm0.25**</td>
<td>41.5\pm4.5*</td>
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<td>4-AP (100 \mu M)</td>
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<tr>
<td>Control</td>
<td>0.305\pm0.03</td>
<td>26.0\pm3.0</td>
<td>0.410\pm0.09</td>
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<tr>
<td>Treated</td>
<td>0.515\pm0.07*</td>
<td>30.5\pm4.0</td>
<td>0.530\pm0.08</td>
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<tr>
<td>Barium (30 \mu M)</td>
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<tr>
<td>Control</td>
<td>0.290\pm0.04</td>
<td>—</td>
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<tr>
<td>Treated</td>
<td>1.080\pm0.15**</td>
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<td>Glibenclamide (1 \mu M)</td>
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<tr>
<td>Control</td>
<td>0.285\pm0.03</td>
<td>27.5\pm4.0</td>
<td>0.410\pm0.07</td>
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<tr>
<td>Treated</td>
<td>0.755\pm0.09*</td>
<td>31.5\pm3.5</td>
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<tr>
<td>Treated</td>
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<td>29.5\pm4.0</td>
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<tr>
<td>IbTX (0.1 \mu M)</td>
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<tr>
<td>Control</td>
<td>0.300\pm0.06</td>
<td>28.0\pm3.5</td>
<td>0.410\pm0.08</td>
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<tr>
<td>Treated</td>
<td>0.910\pm0.25**</td>
<td>51.5\pm7.5**</td>
<td>0.195\pm0.20**</td>
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</table>

Results are represented as mean\pmS.E.M. (n=number of experiments). *p<0.05, **p<0.01 vs. control response in the absence of blockers.

To investigate the role of $K_{ATP}$ on the oscillatory contractions, we added the $K_{ATP}$ channel activator pinacidil (0.1 \mu M) or the $K_{ATP}$ channel blocker glibenclamide (1 \mu M) to the bathing medium after stabilization of the oscillatory contractions. As shown in the Fig. 6, pinacidil rapidly blocked the oscillatory contractions and the tension returned to baseline. After addition of pinacidil, the phenylephrine concentration had to be increased in order to verify similar tension level and the occurrence of oscillatory contractions in the presence of pinacidil. However, pinacidil inhibited the oscillations, even after the vascular tension had been increased (Fig. 6b). On the other hand, glibenclamide, a $K_{ATP}$ blocker, evoked a significant increase in tension from 0.285\pm0.03 to 0.755\pm0.09 g, and a significant reduction in amplitude from 0.410\pm0.07 to 0.180\pm0.06 g. Nevertheless, glibenclamide did not alter the frequency of oscillatory contractions (Fig. 6c). The analyzed parameters (tension, frequency and amplitude) are shown in Figs. 6d, e and f, and the absolute values are presented in Table 1.
experiments, the role of K Ca on oscillatory contractions was studied. The blockade of this type of K Ca with apamin (1 μM) on the oscillatory contractions was significantly reduced (from 0.410±0.08 to 0.195±0.20 g). Finally, the tension was quickly reverted after washing out the preparation with a fresh salt solution. Effects of iberiotoxin on the tension, frequency and amplitude of the oscillatory contractions were also summarized as relative values to control preparations in Figs. 7c, d, e. These absolute values are presented in Table 1.

**DISCUSSION**

Although sinoaortic denervation rats have been useful in the study of the physiological or pathological function of the arterial baroreflex for the control of the arterial pressure, the present study shows that the aorta removed from rats with arterial pressure lability also exhibit oscillatory contractions under phenylephrine stimulus. Our results demonstrate that, although the SAD rats do not become hypertensive, aortas isolated from these rats present oscillatory contractions, which seems to depend on Ca²⁺ and K⁺ currents.

Several types of K⁺ channels have been identified in smooth muscle plasmalemma. These channels play a crucial role in the maintenance of the electrical potential across the surface membrane of smooth muscle cells. The membrane potential of the cell is an important regulator of the contractile force, controlling the entry of Ca²⁺ ions and sometimes also the release of intracellular Ca²⁺, so that changes in the membrane potential lead to changes in intracellular Ca²⁺ and thus contraction. Presumably, oscillatory contractions reflect altered membrane properties that may cause smooth muscle cells to be more excitable than normal. It is well known that both ionic permeability and ion concentration gradients are important determinants of the membrane potential and excitation in the vascular smooth muscle. Also, potassium channels may play an important role in oscillatory contractions, because the frequency of the oscillations is often affected by potassium channel blockers. Although these results demonstrate an influence of potassium channels, they also indicate that potassium channels are not an essential element in the fluctuations of the tension constituting the oscillations, since the majority of the blockers are unable to totally inhibit the oscillatory contractions.

Potassium channels may also be involved through a different mechanism. The non-selective inhibition of potassium channels with tetraethylammonium (TEA) has consistently been shown to promote oscillatory contractions in smooth muscle preparations. Corroborating these authors, in the present study TEA has potentiated the oscillatory contractions in SAD rat aortas, increasing both the amplitude and the frequency of the oscillations. One possible explanation for this effect is a TEA-induced decrease in membrane conductance, which should promote depolarization and muscle contraction. For this reason, the role of some potassium channels and their regulation in oscillatory contractions has been strongly suggested. Moreover, TEA could be acting in a way that differs from the action of the K⁺ channels. One possibility is that TEA promotes oscillatory contractions through the induction of gap junctions. One suggested mechanism is that TEA induces the formation of gap junctions and there-

**Fig. 6. Effects of Pinacidil and Glibenclamide on the Oscillatory Contractions in the SAD Rat Aortic Rings**

(a) Control preparations showing typical rhythmic contractions (n=5) induced by phenylephrine (arrows). (b) A representative tracing illustrating the effect of pinacidil (0.1 μM) on the oscillatory contractions. The symbol (▾) indicates phenylephrine addition able to increase the vascular tension to the same values obtained before pinacidil (n=5). (c) A representative tracing illustrating the effect of glibenclamide (gliben, 1 μM) on the oscillatory contractions (n=7). (d, e, f) Data showing the effect of glibenclamide on the tension, frequency and amplitude of oscillatory contractions. Data are mean±S.E.M. of seven experiments. * Denotes difference between glibenclamide and control (p<0.05).

**Fig. 7. Effects of the K Ca Channel Blockers on Oscillatory Contractions in the SAD Rat Aortic Rings**

(a) Representative tracing illustrating the effect of apamin (1 μM) on the oscillatory contractions (n=4). (b) A representative tracing illustrating the effects of iberiotoxin (IbTX, 0.1 μM) on the oscillatory contractions (n=6). (c, d, e) Data showing the effect of iberiotoxin on the tension, frequency and amplitude of the oscillatory contractions. Data are mean±S.E.M. of seven experiments. * Denotes difference between iberiotoxin and control (p<0.01).

**K Ca (KCa) on Oscillatory Contractions** In the next series of experiments, the role of K Ca on oscillatory contractions was examined. Figure 7a shows the effect of apamin, which acts as selective blocker of low-conductance, K Ca (SK) channels. The blockade of this type of K Ca with apamin (1 μM) did not cause any alteration in the oscillatory contractions (n=4). We also tested the effect of iberiotoxin (0.1 μM), a selective blocker of large-conductance K Ca (BK) channels. As clearly shown in Fig. 7b, iberiotoxin (n=5) considerably increased the tension (from 0.300±0.06 to 0.910±0.25 g) and the frequency of the oscillatory contractions (from 28.0±3.5 to 51.5±7.5 counts for 5 min). Despite the increased tension and frequency, the amplitude of the oscillatory contractions was significantly reduced (from 0.410±0.08 to 0.195±0.20 g). Finally, the tension was quickly reverted after washing out the preparation with a fresh salt solution. Effects of iberiotoxin on the tension, frequency and amplitude of the oscillatory contractions were also summarized as relative values to control preparations in Figs. 7c, d, e. These absolute values are presented in Table 1.
fore promotes oscillatory contractions.24,25,28)

Our present findings also show that verapamil quickly inhibits the oscillatory contractions and abolishes the tonic phase of the contraction elicited by membrane depolarization as a result of TEA effects. The Ca\(^{2+}\) influx through voltage-gated Ca\(^{2+}\) channels seems to play a significant role in the generation of oscillatory contractions in the SAD rat aortas. Therefore, in our experiments, oscillatory contractions seem to be dependent on extracellular Ca\(^{2+}\), because low concentrations of verapamil totally block the oscillations.

K\(_{\text{v}}\) channels belong to the superfamily of voltage-gated channels and they are activated by depolarization. In the arterial smooth muscle there is evidence that these channels contribute to resting potassium conductance.20) In some types of smooth muscle as well as the guinea-pig portal vein29) and guinea-pig urinary bladder,14) 4-AP dramatically increases the frequency of oscillatory contractions. Although these channels can present physiological particularities in different smooth muscle preparations, the effects of 4-AP on the oscillatory contractions in isolated aortic rings of SAD rats are mild. Although the concentration used in this study is able to block K\(_{\text{v}}\),30) 4-AP increased the tension only, but it did not alter the frequency and amplitude of the oscillations.

The K\(_{\text{v}}\) channel is expressed in the vascular smooth muscle cells, suggesting its potential role in tonus regulation.19) Vascular K\(_{\text{v}}\) channels are very sensitive to barium. It has been shown at single channel and at intact tissue levels that a low concentration (30 \(\mu\)M) of barium is a well known selective inhibitor of the vascular smooth muscle cell K\(_{\text{v}}\) channels.31—33) It is known that the closure of potassium channels produces membrane depolarization and muscle contraction. In our study, the treatment with barium chloride caused both an increase in the basal tension and an interruption of the oscillatory contractions, showing that in the aortic rings of SAD rats there is an important involvement of K\(_{\text{v}}\) channel, at least in the oscillatory contractions. Despite the intense tonic contraction evoked by K\(_{\text{v}}\) blockade, the effects of barium were reverted and the tension returned to baseline immediately after washout with fresh Krebs solution. Alternatively, the sustained tonic effects of barium might have masked the role of K\(_{\text{v}}\) channel on the oscillatory contractions. One hypothesis is that the increase in tonic evoked by barium might impair the relaxation phase of the oscillatory contraction cycles. If the same happens, in our study we could be underestimating the role of K\(_{\text{v}}\) channels in the oscillatory contractions.

Another important means of potassium efflux is the K\(_{\text{ATP}}\) channels. They are characterized by their metabolic sensitivity, and they have been identified and named after their sensitivity to inhibition by intracellular ATP. K\(_{\text{ATP}}\) channels are actively involved in the regulation of rhythmic cellular activities. The activation of K\(_{\text{ATP}}\) channels also inhibits oscillatory contraction of smooth muscles in the guinea-pig mesotubarium.34) Using the transgenic approach, Kakkar and colleagues (2006),35) demonstrated in a recent article, that gene deletion of K\(_{\text{ATP}}\) does not interfere with the vasospasm in mouse arteries, excluding the role of K\(_{\text{ATP}}\) in the artery vasospasm exhibited by the coronary artery. Among the K\(_{+}\) channel modulators tested in the present study, inhibition of oscillatory contractions was most pronounced by pinacidil, a drug known for activating K\(_{\text{ATP}}\) channels. In fact, our results indicate that oscillatory contractions are related to K\(^{+}\) currents sensitive to this channel modulator. Effects of K\(_{\text{ATP}}\) channel blockade were evident, since glibenclamide decreased the amplitude of oscillatory contractions. Therefore, the maintenance of the oscillations seems to depend on the K\(^{+}\) current through the K\(_{\text{ATP}}\) channels.

The cytosolic Ca\(^{2+}\) concentration can modulate some ion channels, such as Ca\(^{2+}\)-activated K\(^{+}\) channels (K\(_{\text{Ca}}\)). The involvement of K\(_{\text{Ca}}\) channels in the oscillatory vascular contractions of many vascular tissues is uncertain. In a study by Lamb and Webb (1989),13) iberiotoxin and apamin had no effects on the oscillatory contractions in the tail arteries from SHR, although the altered K\(^{+}\) conductance played an important role in those hypertensive tissues. In another study, neither apamin, pinacidil nor glibenclamide affected the rhythmic activity of rat mesenteric arteries induced in vitro with norepinephrine.36)

In the present study the blockade of SK\(_{\text{Ca}}\) channels with apamin did not alter the pattern of the oscillatory contractions. Therefore, we exclude the contribution of SK\(_{\text{Ca}}\) channels to the regulation of oscillatory contractions and tension adjustment in SAD aortic rings. These results suggest that SK\(_{\text{Ca}}\) might not be involved in the oscillatory contractions of the aorta of SAD rats.

Structurally, another K\(_{\text{Ca}}\) channel is distinguished: the large conductance Ca\(^{2+}\)-activated K\(^{+}\) channel (BK\(_{\text{Ca}}\)), which is blocked by iberiotoxin. BK\(_{\text{Ca}}\) is thought to play an important physiological role in the repolarization phase of the action potential. In earlier studies, it has been demonstrated that BK\(_{\text{Ca}}\) plays an important role in controlling oscillatory contractions in some smooth muscle preparations, such as the rat basilar artery,11) rat mesenteric artery,10,12) and rabbit ear artery.7) In our hands, the inhibition of BK\(_{\text{Ca}}\) with iberiotoxin altered the oscillatory contractions, increasing their frequency and decreasing their amplitude. Effects of BK\(_{\text{Ca}}\) channels blockade were evident from the pronounced increase in both the oscillation frequency and tension in the presence of iberiotoxin. Therefore, due to the important role of BK\(_{\text{Ca}}\) in the maintenance of the resting membrane potential, it can affect the oscillations in aortas of SAD rats when it is blocked.

In conclusion, we have demonstrated that SAD rat aortas display endothelium-independent rhythmic contractions when stimulated to contract with phenylephrine. We have shown that K\(_{\text{ATP}}\) and BK\(_{\text{Ca}}\) channels play a dominant role in the regulation of the oscillatory contractions of the SAD rat aortas. Furthermore, a possible contribution of barium-sensitive K\(_{\text{v}}\) channels to this oscillatory behavior has also been implicated and we do not discard the influence of K\(_{\text{v}}\) channels on the oscillatory contractions.

Acknowledgments This work was supported by FAPESP and CNPq.

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