Antihypertensive Action of Amiodarone in Spontaneously Hypertensive Rats

Valdo José Dias da Silva, Publio Cesar Cavalcante Viana, Rodrigo de Melo Alves, Helio Cesar Salgado, Nicola Montano, Rubens Fazan, Jr

Abstract—The antihypertensive effect of amiodarone was investigated in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). The SHR and WKY were treated with amiodarone (1 mg/mL PO) or tap water (control) for 20 weeks. The indirect arterial pressure (AP) was monitored weekly using the tail-cuff method. At the end of the 20th week, the direct AP was measured, and the systolic AP and pulse interval time series were submitted to autoregressive spectral analysis. In addition, cardiac baroreflex sensitivity and left ventricular weight were evaluated as well. The indirect AP was reduced 1 week after the beginning of amiodarone treatment. The direct mean AP and pulse interval were, respectively, 135±8 mm Hg and 191±3 ms in SHR treated with amiodarone (187±8 mm Hg and 156±7 ms in control SHR, P<0.05) and 87±3 mm Hg and 207±8 ms in WKY treated with amiodarone (105±8 mm Hg and 174±4 ms in control WKY, P<0.05). In SHR treated with amiodarone, the low-frequency oscillations of AP were lower (8.5±1.2 mm Hg versus 14.4±2.9 mm Hg in control SHR, P<0.05), whereas the reflex bradycardia was higher (0.84±0.12 ms/mm Hg versus 0.32±0.22 ms/mm Hg in control SHR, P<0.05). The left ventricle weight was also smaller in SHR treated with amiodarone (2.94±0.12 mg/g versus 3.45±0.24 mg/g in control SHR, P<0.05). In WKY, amiodarone induced similar changes as in SHR, except for a lack of effect in the left ventricle weight. These data indicate that amiodarone has an antihypertensive action in SHR that is associated with a reduction in vasomotor sympathetic modulation, an increase in vagal cardiac baroreflex sensitivity, and a decrease in cardiac hypertrophy. (Hypertension. 2001;38[part 2]:597-601.)

Key Words: antiarrhythmic agent ▪ sympathetic nervous system ▪ autonomic nervous system ▪ baroreceptors

Art erial hypertension is the most common cardiovascular disorder, causing several end-organ lesions. As a consequence of the hemodynamic overload, a cardiac hypertrophy may be commonly associated with hypertension. Moreover, sympathetic overactivity and vagal baroreflex depression may also be present. All these effects can be associated with the life-threatening cardiac arrhythmias and sudden death in hypertensive patients.1

Several clinical trials have demonstrated the relative efficacy and safety of the chronic oral administration of amiodarone in preventing life-threatening cardiac arrhythmias such as ventricular tachycardia and ventricular fibrillation.2 The mechanism of the antiarrhythmic action of amiodarone is complex and not completely understood. Amiodarone is an antiarrhythmic that prolongs cardiac depolarization and refractory period.3 Moreover, Na+ and Ca2+ channel blockade and antiadrenergic properties have also been reported for amiodarone.3,4 Additionally, an antityroidian effect during long-term amiodarone administration has been described.5 Some of the mechanisms of amiodarone, such as sympathetic inhibition and Ca2+ channel blockade, are potentially antihypertensive. Nevertheless, there is no report in the literature addressing this issue. Therefore, in the present study, we evaluated the arterial pressure (AP) levels in young adult spontaneously hypertensive (SHR) and age-matched normotensive Wistar-Kyoto rats (WKY) treated chronically (20 weeks) with amiodarone. We also evaluate the AP and HR variability spectra and baroreflex sensitivity in SHR and WKY treated with amiodarone. The relative left ventricular weight was also measured to evaluate the cardiac hypertrophy.

Methods

Sixteen-week-old male SHR (n=9) or WKY (n=10) received a solution of amiodarone (1 mg/mL) for 20 weeks. The rats drank approximately 30 mL/d of this solution, receiving ~30 mg of amiodarone daily. This dosage has been used in different pharmacokinetic and pharmacodynamic studies in rats to evaluate its cardiovascular effects.6 Age-matched SHR (n=8) and WKY (n=6) received only tap water during the same period. Indirect AP and pulse interval (PI) of all animals were weekly monitored by means of...
the tail-cuff method (Blood Pressure Meter, Letica Scientific Instrumentation).

At the end of the 20-week period, under sodium pentobarbital (40 mg/kg IP) anesthesia, arterial and venous catheters were implanted into the femoral artery and vein for direct measurement of AP and drug administration, respectively. After the surgical procedures, the animals recovered in individual cages for at least 24 hours.

One day after the surgical procedures, without the effect of anesthesia, the arterial catheter was connected to a pressure transducer (Statham P23b, Hato Rey), and the signal was amplified and continuously sampled (1000 Hz) on a microcomputer equipped with a 12-bit analog-to-digital board (CAD12/36 Lynx Eletrônica). During the experiment, silence was maintained inside the room to minimize the influence of stress on AP and PI.

After at least 30 minutes of basal pulsatile AP recording, the animals received alternating intravenous bolus injections of phenylephrine (0.25 to 8 µg/kg) and sodium nitroprusside (1 to 32 µg/kg) to change AP and assess baroreflex sensitivity. At the end of the experiments, the animals were euthanized with ether inhalation and had their hearts removed. The left ventricles were dissected and weighted.

AP recordings were processed by a computer software that applies an algorithm to detect cycle-to-cycle inflection points of a periodic waveform, determining beat-by-beat values of systolic and diastolic pressures. Beat-by-beat PI series were also generated by measuring the length of time between adjacent diastolic pressure readings. The overall variability of systolic AP (SAP) and PI was assessed by means of variance of the time series. The SAP and PI variabilities in the frequency domain were assessed by autoregressive spectral analysis as described elsewhere. Briefly, a modeling of the oscillatory components presented in stationary segments of beat-by-beat time series of SAP and PI was calculated based on Levinson-Durbin recursion, with the order of the model chosen according to Akaike’s criterion. This procedure allows an automatic quantification of the center frequency and power of each relevant oscillatory component present in the time series. The oscillatory components were labeled as low (LF) or high frequency (HF) when their central frequencies were in a band of 0.20 to 0.75 or 0.75 to 2.50 Hz, respectively. The power of LF and HF components of PI variability are also expressed in normalized units, obtained by calculating the percentage of the LF and HF variability with respect to the total power after subtracting the power of the very-low-frequency component (frequencies <0.20Hz). The normalization procedure tends to minimize the effect of the changes in total power on the absolute values of LF and HF components.

Baroreflex sensitivity was quantified by the slope of the regression line obtained by best-fit points relating changes in AP and SAP with respect to their baseline values.

Data are expressed as mean±SEM. A multivariate ANOVA for repeated measures followed by a Tukey’s multiple comparison test was applied on data obtained from tail-cuff blood pressure monitoring. For all other data, a 2-way ANOVA followed by a Tukey’s multiple comparison test was performed to evaluate the effects of the oscillatory component present in the time series. The oscillatory components were labeled as low (LF) or high frequency (HF) when their central frequencies were in a band of 0.20 to 0.75 or 0.75 to 2.50 Hz, respectively. The power of LF and HF components of PI variability are also expressed in normalized units, obtained by calculating the percentage of the LF and HF variability with respect to the total power after subtracting the power of the very-low-frequency component (frequencies <0.20Hz). The normalization procedure tends to minimize the effect of the changes in total power on the absolute values of LF and HF components.

Baroreflex sensitivity was quantified by the slope of the regression line obtained by best-fit points relating changes in AP and PI with respect to their baseline values.

Data are expressed as mean±SEM. A multivariate ANOVA for repeated measures followed by a Tukey’s multiple comparison test was applied on data obtained from tail-cuff blood pressure monitoring. For all other data, a 2-way ANOVA followed by a Tukey’s multiple comparison test was performed to evaluate the effects of the changes in total power on the absolute values of LF and HF components.

Results

The time course of indirect SAP and PI during the 20 weeks of treatment with amiodarone is shown in Figure 1. Systolic AP was reduced after the first week of treatment in SHR, and this reduction was accompanied by remarkable bradycardia. Hypotension associated with marked bradycardia was also observed in WKY. The direct AP and PI are shown in the Table. Chronic treatment with amiodarone in SHR promoted a significant reduction of systolic, diastolic, and mean AP associated with a marked bradycardia compared with the values in control SHR. In WKY, amiodarone produced a smaller hypotensive effect accompanied by bradycardia as well.

The spectral pattern of PI and SAP variabilities presented LF and HF oscillatory components in all groups of animals studied (Figure 2). No difference was observed in the spectral pattern of PI variability between SHR and WKY that received only tap water; nevertheless, the LF component of SAP variability was greater in SHR than in WKY treated with amiodarone (Figure 2). The values are expressed as mean±SEM and represent the average for consecutive periods of 2 weeks. The arrow indicates the beginning of amiodarone administration. Notice the significant reduction in SAP and the significant increase in PI after the beginning of amiodarone treatment (SAP: P<0.01 vs WKY that received tap water; Fstrain=115.06, P<0.001; Ftreatment=28.06, P<0.01; Ftime=10.12, P<0.01; Finteraction=5.08, P<0.01; and PI: Fstrain=1.06, P=0.304; Ftreatment=16.06, P<0.01; Ftime=9.10, P<0.01; and PI: Finteraction=6.06, P<0.01).

Figure 1. Time course of SAP (upper panel) and PI (lower panel) 4 weeks before and during 20 weeks of chronic oral administration of amiodarone to normotensive WKY (○) and SHR (●). The values are expressed as mean±SEM and represent the average for consecutive periods of 2 weeks. The arrow indicates the beginning of amiodarone administration. Notice the significant reduction in SAP and the significant increase in PI after the beginning of amiodarone treatment (SAP: P<0.01 vs WKY that received tap water; Fstrain=115.06, P<0.001; Ftreatment=28.06, P<0.01; Ftime=10.12, P<0.01; Finteraction=5.08, P<0.01; and PI: Fstrain=1.06, P<0.01; Ftime=9.10, P<0.01; Finteraction=6.06, P<0.01).

The reflex bradycardia induced by the increase in AP in response to phenylephrine was significantly higher in both SHR (0.84±0.12 versus 0.32±0.02 ms/mm Hg, P<0.05) and WKY (1.59±0.09 versus 1.22±0.15 ms/mm Hg, P<0.05) treated with amiodarone (Figure 3). The reflex bradycardia in
SHR treated with amiodarone was also significantly different from WKY rats treated with amiodarone (0.84 ± 0.12 ms/mm Hg versus 1.59 ± 0.09 ms/mm Hg in WKY rats, P < 0.05). The reflex tachycardia induced by the decrease in AP in response to sodium nitroprusside was not affected by the treatment with amiodarone in both SHR (0.73 ± 0.29 versus 0.56 ± 0.21 ms/mm Hg) and WKY (1.10 ± 0.38 versus 0.87 ± 0.09 ms/mm Hg).

The relative left ventricle weight of SHR treated with amiodarone was significantly lower than that of SHR that received only tap water (2.94 ± 0.12 versus 3.45 ± 0.24 mg/g, P < 0.05). In WKY, amiodarone did not affect the relative left ventricle weight (1.97 ± 0.05 versus 2.02 ± 0.06 mg/g).

**Discussion**

The present study shows that chronic oral treatment with amiodarone in SHR causes a significant reduction in AP accompanied by a marked bradycardia. These effects were associated with a reduction in the LF oscillation of AP.
An increase in the vagal component of baroreflex sensitivity was observed in both SHR and WKY after chronic amiodarone. This effect could be related to (1) a possible direct excitatory effect on arterial baroreceptor nerve endings, possibly related to its Na\(^+\),K\(^+-\)ATPase blocking properties of the drug\(^3\), (2) a sympatholytic action blunting the sympathetic restraint of the baroreflex control of heart rate\(^4\); (3) a central facilitatory effect on neural structures integrating the baroreflex arch, such as the nucleus tractus solitarius\(^6\); and (4) a reduction in cardiac hypertrophy, because there is evidence indicating a direct relationship between cardiac hypertrophy and baroreflex.\(^7\) Nevertheless, this last hypothesis should be restricted to SHR.

Amiodarone partially prevented the cardiac hypertrophy in SHR, an effect possibly related to a reduction in afterload due to the fall in AP. However, a direct effect of amiodarone reducing the sympathetic drive to the heart and/or blocking Ca\(^{2+}\) channels cannot be ruled out because these mechanisms have been also involved in the genesis of cardiac hypertrophy.\(^8\) An acute sympathetic overdrive acting on a hypertrophied ventricular myocardium has been implicated as a trigger for life-threatening cardiac arrhythmias, which are commonly observed in hypertensive patients.\(^1\) It should be remembered that both increased vagal modulation and increased BRS have been associated with a reduced risk of life-threatening arrhythmias and sudden death.\(^9\) Therefore, the vagotonic and sympatholytic properties of amiodarone observed in the present study may bring some insight into the antiarrhythmic and antihypertensive properties of this drug in hypertensive subjects.

In conclusion, our findings indicate that chronically administered oral amiodarone has antihypertensive properties associated with decreased vascular sympathetic drive, increased cardiac vagal baroreflex, and reduction of cardiac hypertrophy. These effects should be taken into account in the treatment of cardiac arrhythmias associated with hypertensive states.

References


Antihypertensive Action of Amiodarone in Spontaneously Hypertensive Rats
Valdo José Dias da Silva, Publico Cesar Cavalcante Viana, Rodrigo de Melo Alves, Helio Cesar Salgado, Nicola Montano and Rubens Fazan, Jr

*Hypertension*. 2001;38:597-601
doi: 10.1161/hy09t1.096187

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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/38/3/597

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/