Opposite effects of iv amiodarone on cardiovascular vagal and sympathetic efferent activities in rats

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Methods

Experimental Preparation

All experiments were performed according to the Guiding Principles for Research Involving Animals and Human Beings endorsed by the American Physiological Society.

Sudden death represents the major cause of mortality in the Western countries. Sympathetic hyperactivity has been implicated in triggering life-threatening arrhythmias, while, on the other hand, vagal activation may partly play a protective role (18, 21, 23). Thus a drug characterized by a mechanism of action able to reduce sympathetic activity and increase vagal activity might be of great benefit in preventing ventricular fibrillation and tachyarrhythmias. During the past decade, randomized clinical trials have investigated the ability of several antiarrhythmic drugs to reduce sudden death in patients at high risk of arrhythmias (9, 24). Apart from β-blockers, no other agent has been conclusively found to reduce mortality (25). However, a recent meta-analysis (1) indicated that amiodarone reduces the rate of arrhythmic/sudden death in high-risk patients with myocardial infarction or congestive heart failure. Amiodarone is a widely used antiarrhythmic drug that lowers heart rate, reduces premature ventricular contractions, and prevents ventricular fibrillation (5, 14, 15). This action is likely to occur through several antiarrhythmic mechanisms, such as a Na+ channel blockade, Ca2+ channel blockade, prolongation of cardiac repolarization and effective refractory period (13), and a peripheral antiadrenergic effect (8). However, no information is available on the possible direct central effects on autonomic neural mechanisms.

In this study we report that, in decerebrate unanesthetized, spontaneously breathing rats, intravenous amiodarone increased vagal while reducing sympathetic preganglionic activity. The neural changes were associated with bradycardia and hypotension and were still evident in the absence of baroreflex mechanisms, suggesting a direct effect of this drug on central neural structures. These combined vagotonic and sympatholytic effects may contribute to the powerful antiarrhythmic action of amiodarone.

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Experiments were performed on 25 normotensive Sprague-Dawley rats (350–450 g). Rats were anesthetized with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). A midline cervical incision was performed, and both common carotid arteries were isolated and occluded by ligature. Polyethylene catheters (PE-50) were inserted into the right common carotid artery and the right femoral vein. The trachea was cannulated (PE-120) using a T tube that was connected on one side to an end-tidal CO₂ analyzer (Capstar, Stoelting, Ardmore, PA).

Subsequently all rats were placed on a stereotaxic apparatus. A midsagittal incision was performed, and the skull was exposed. After removal of the parietal bones, a bilateral midcollicular transection was performed and the forebrain was removed by suction. The accuracy of the transection was confirmed by postmortem examination. Decerebration allowed the rest of the experiment to be carried out without the depressive influence of anesthesia on neural structures.

After a xifo-umbilical incision, the aorta and the inferior vena cava were isolated and pneumatic cuffs were placed around each vessel to induce controlled increases (inflation of the aortic cuff) and decreases (inflation of the vena cava cuff) in arterial blood pressure (AP).

All animals were spontaneously breathing with a respiratory rate between 56 and 120 breaths/min and an end-tidal CO₂ between 3.5 and 5.5%.

In all animals, electrocardiogram, AP, and endotracheal pressure were recorded. A bipolar electrode was used for neural recordings. All signals were acquired and stored on a personal computer equipped with an analog-to-digital board at a sampling rate of 12 kHz.

**Neural Recordings**

**Vagal recording.** The left cervical vagus nerve was isolated from the surrounding tissues and peripherally cut. The nerve sheath was removed, and the nerve was split to isolate vagal cardiovascular preganglionic fibers. Vagal cardioinhibitory fibers were characterized by a discharge synchronized with the expiratory phase of respiration and responsive to baroreflex mechanisms, namely reducing or increasing their discharge during, respectively, hypotension or hypertension (3). An example of vagal efferent recording is shown in Fig. 1A.

**Sympathetic recording.** The left cervical sympathetic trunk was isolated from the surrounding tissues and peripherally cut. The nerve sheath was removed, and the nerve was split to isolate sympathetic cardiovascular preganglionic fibers. Sympathetic cardiovascular fibers were characterized by their response to baroreflex mechanisms, namely reducing or increasing their discharge during, respectively, hypertension or hypotension. An example of sympathetic efferent recording is shown in Fig. 1B.

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**Fig. 1.** A: raw vagal nerve activity (VNA), integrated VNA (int VNA), arterial pressure (AP), and R-R interval (RR) in baseline condition (Control), aortic constriction (AoC), and inferior vena cava constriction (IVCC) in a rat. Increase in AP induced by AoC was associated with increase in VNA and bradycardia. Conversely AP reduction induced by IVCC was associated with a decrease in VNA and tachycardia. B: raw sympathetic nerve activity (SNA) and integrated SNA (int SNA) in a rat. Increase in AP induced by AoC was associated with decrease in SNA and tachycardia. Conversely AP reduction induced by IVCC was associated with an increase in SNA and tachycardia.
Experimental Protocol

One hour after nerve filament isolation, recordings were continuously performed from 10 min before to 1 h after an intravenous dose of 50 mg/kg (dissolved in 1 ml/kg of vehicle) of amiodarone. Vagal fibers were recorded in rats with intact baroreflex (n = 7). In four of seven animals recordings were also performed after barodenervation at the end of the experiment. Sympathetic fibers were recorded in two groups: one with intact baroreflex (n = 8) and the other with barodenervation (n = 5). Barodenervation was performed by bilateral section of the aortic depressor nerve and was confirmed by the absence of reflex changes in autonomic nerve activities and heart rate after increases or decreases in AP.

Effects of Atropine

To quantify the role of vagal nerve activity in inducing bradycardia, in a separate group of animals (n = 5), atropine (5 mg/kg iv) was given 20 min after intravenous administration of amiodarone. These animals underwent a surgical procedure and experimental protocol similar to the one followed in the other groups, including left cervical vagus nerve section but no nerve recordings were performed.

Data Analysis

The neural signal was fed into a preamplifier (Grass RPS 107, Quincy, MA) and filtered using a 30- to 3,000-Hz bandwidth. A counted neural activity signal was obtained by calculating the number of spikes over a temporal basis of 10 ms using an automatic digital program based on a threshold detection. The threshold level was set to avoid the background noise. This stepwise signal was filtered at 2 Hz. This procedure does not affect the mean value of neural activity but allows us to obtain values per second adjusted for the heart rate changes. R-R interval, mean AP (MAP), and vagal or sympathetic neural activity were measured at 1 min before and 1, 5, 10, 20, 30, 40, 50, and 60 min after intravenous amiodarone administration.

Statistical Analysis

Data are expressed as means ± SE. To evaluate the effects of the treatment on repeated measurements of all data, a one-way ANOVA for repeated measures followed by a Tukey’s multiple comparison test was performed using the software SPSS 7.5 for Windows (SPSS, Chicago, IL). Differences were considered significant when P < 0.05.

RESULTS

Vagal Recordings

Results are summarized in Fig. 2, left. In baseline conditions, the average values of vagal nerve activity, R-R interval, and MAP were, respectively, 16.4 ± 3.3

Fig. 2. Left: time course of VNA, R-R interval, and mean arterial pressure (MAP) before and after intravenous amiodarone (indicated by the broken line) recorded in the group of 7 rats. Right: time course of SNA, R-R, interval and MAP before and after intravenous amiodarone recorded in the group of 8 rats. The values are expressed as means ± SE. The marked bradycardia and hypotension induced by amiodarone were associated with a progressive increase of VNA after drug administration that became significant after 20 min. SNA showed an abrupt but transitory increase in SNA followed by a significantly reduction after 20 min. *P < 0.01 vs. baseline.
spikes/s, 150 ± 13 ms, and 113 ± 8 mmHg. Vagal activity was already increased 1 min after amiodarone administration, reaching a significant difference after 20 min (260 ± 131%; P < 0.01) up to the end of the observation period. R-R interval underwent an increase that became significant after 5 min (112 ± 42%; P < 0.01) up to the end of the recording session. MAP decreased significantly after the first minute after amiodarone administration (21 ± 9%; P < 0.01) and remained significantly lower during the rest of the experiment.

In four animals in which a barodenervation was performed 60 min after amiodarone administration, vagal activity remained significantly higher compared with baseline conditions (325 ± 107%; P < 0.01).

**Sympathetic Recordings**

Results are summarized in Fig. 2, right. Before amiodarone, the average values of sympathetic nerve activity, R-R interval, and MAP were, respectively, 24.8 ± 5.7 spikes/s, 190 ± 17 ms, and 115 ± 4 mmHg.

A short-lasting increase in sympathetic activity was detectable during the first minute after amiodarone administration (158 ± 83%; P < 0.01) but was soon replaced by a progressive decrease reaching statistical significance at 20 min (36 ± 17%; P < 0.01) and for the rest of the observation period. Despite the increase in sympathetic activity 1 min after amiodarone, the R-R interval increased and became significant at 5 min (+61 ± 10%; P < 0.01) up to the end of the experiment. MAP was already significantly decreased (−32 ± 7%; P < 0.01) 1 min after amiodarone administration and remained significantly lower for the whole experiment.

In the group of animals in which barodenervation was performed at the beginning of the experiment (Fig. 3), the initial increase in sympathetic activity was not observed, whereas a decrease in nerve activity was similarly present throughout the whole experiment, reaching statistical significance after 20 min (41.1 ± 12%; P < 0.01). The barodenervated group showed no differences at baseline for MAP while having a higher SNA and R-R interval.

**Effect of Atropine**

In the group of animals in which muscarinic blockade was performed 20 min after amiodarone administration, the drug-induced bradycardia was reduced by 33 ± 9% (P < 0.05), whereas MAP was not significantly affected.

**DISCUSSION**

Our study provides the first evidence that amiodarone increases the discharge of vagal efferent fibers while reducing the sympathetic outflow discharge. These autonomic changes were accompanied by a significant decrease in heart rate and AP and were preserved after barodenervation, suggesting a central effect of the drug.

Vagal and sympathetic nerve filaments were likely to include an efferent activity largely participating in cardiovascular neural regulation, according to functional criteria largely accepted in various human and animal experimental conditions (19).

These data are consistent with previous observations in conscious rats (7) in which, after acute administration of amiodarone, bradycardia and hypotension induced by amiodarone were associated with a progressive reduction of SNA that became significant after 20 min. Note the absence of the transitory increase of SNA in barodenervated animals compared with the intact ones. *P < 0.05 vs. baseline. #P < 0.05 barodenervated vs. intact.
mechanism seems involved in the abrupt increase in sympathetic activity in concomitance to AP decrease observed 1 min after amiodarone administration, because it was abolished by the barodenervation procedure. Therefore, this increase in cardiac vagal activity may participate in determining the reduction in heart rate induced by the drug. However, although it has been reported that amiodarone crosses the blood-brain barrier (22), to our knowledge, no data are available regarding a possible specific site of action at the central nervous system level.

The bradycardia observed after intravenous amiodarone (14) has been attributed to different mechanisms, including direct Na\(^+\) and Ca\(^{2+}\) channel blocking properties that depress the automaticity of sinus node (10, 13), non-competitive β-adrenergic blockade, and reserpine-like sympatholytic action (2, 8, 13). Controversial evidence is present in the literature regarding a possible effect of amiodarone on cardiac vagal modulation. Indeed, in vivo studies on muscarinic blockade reported that amiodarone does not affect (2) or slightly reduces (11) vagal modulation, whereas in vitro studies indicated that this drug might possess a vagolytic effect because it is able to block cardiac muscarinic receptors (20) and inhibit the acetylcholine-dependent K\(^+\) current in atrial cells (4). However, our data seem to demonstrate a direct excitatory effect of amiodarone on vagal efferent discharge that may importantly contribute to the induced bradycardia. Indeed, sinus node muscarinic blockade with atropine was able to increase heart rate by >30%.

Amiodarone-induced hypotension may be due to a variety of mechanisms such as a vascular sympatholytic effect, probably via a reserpine-like action (15) or an α-adrenergic blocking action (7). In addition, a direct effect on vascular smooth muscle cells due to the Ca\(^{2+}\) channel blocking properties of the drug (1) should be taken into account. Moreover, the vehicle per se, containing polysorbate-80, could exert a hypotensive effect (10). However, studies from our laboratory were not able to show any hypotensive effect of vehicle containing polysorbate-80 in conscious rats (7).

### Study Limitations

A major limitation of our study is that we did not evaluate a dose-response relationship for amiodarone. However, we selected a dose that has been commonly used in rats to evaluate its cardiovascular effects (15, 16, 22). Interestingly, in these studies, plasmatic dosing of amiodarone after intravenous injection ranges from ~45 μg/ml at the early time to 5 μg/ml at 60 min (22), which is very similar to that found in humans after an acute dose of intravenous amiodarone (27).

Although we hypothesized a central effect to explain the observed changes in autonomic efferent activities, we did not measure amiodarone brain concentration and distribution. However, amiodarone has been shown to be detectable in the rat brain and to reach the highest concentration 20–30 min after intravenous administration (22). Therefore, the timing of brain distribution of amiodarone seems consistent with the observed modifications in vagal and sympathetic nerve discharges that became significant after 20 min of drug administration.

### Clinical Implications

Acute sympathetic excitation acting on a dysfunctional ventricular myocardium has been implicated as a trigger for life-threatening cardiac arrhythmias in patients with coronary artery disease, heart failure, and hypertension (18, 21, 23). Accordingly, increased vagal modulation and enhanced baroreflex sensitivity have been associated with a reduced risk of life-threatening arrhythmias and sudden death (21). Pharmacological interventions aimed to reduce the cardiac effect of sympathetic activation and/or to increase cardiac vagal modulation might have beneficial effects in preventing cardiac arrhythmias (1, 3, 21). It has been reported that while vagally mediated paroxysmal atrial fibrillation is preferentially observed in the absence of detectable heart disease, sympathetically mediated atrial fibrillation is observed in the presence of any heart disease, inducing a vagal withdrawal (26).

Lombardi et al. (17) recently described that patients with early atrial fibrillation recurrences are characterized by an enhanced sympathetic and reduced vagal modulation to the sinus node, suggesting that an alteration in the sympathovagal balance may contribute to the electrical remodeling involved in the genesis and maintaining of atrial fibrillation. Within this latter scenario, amiodarone would be a candidate drug to exert beneficial autonomic effects on the heart.

In conclusion, our data indicate that amiodarone possesses vagotonic and sympatholytic properties. We suggest that these effects may contribute to the powerful antiarrhythmic action of acute amiodarone administration.

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