Constricting and Stiffening Action of Atropine on Aortic Response to Angiotensin in Dogs

EDMUNDO CABRERA, JAIME LEVENSON, RICARDO ARMENTANO, JUAN BARRA, RICARDO PICHET, AND ALAIN SIMON

SUMMARY The elasticity of the thoracic aorta was studied in nine dogs instrumented with a pressure microtransducer and two ultrasonic crystals diametrically fixed in the adventitia. Systolic and diastolic changes in pressure and diameter were used to calculate Peterson and incremental elastic moduli. Acute hypertension was induced by infusions of angiotensin performed 1) during the control period, 2) after propranolol (1.5 mg/kg), 3) after atropine (0.2 mg/kg), and 4) after propranolol plus atropine. Absolute and percent variations of mean diameter were correlated to pressure in the control period and after autonomic blockade (p<0.01). The slopes of these correlations were not different between propranolol and control groups, but were lower with atropine (p<0.01) and with atropine plus propranolol (p<0.001) than in the control period. Correlations were also found between Peterson and incremental elastic moduli and mean pressure in the control period and after blockade (p<0.001). No differences of slopes existed between propranolol and control groups, but the slope of the correlation relative to the incremental elastic modulus was higher with atropine than in the control period (p<0.05), and the slopes of the correlations relative to the Peterson and the incremental elastic moduli were respectively higher with atropine plus propranolol than in the control period (p<0.05, p<0.05). Thus, atropine decreased the distention and increased the stiffness of the aorta in response to acute angiotensin-mediated hypertension. (Hypertension 11 [Suppl I]: I-103-I-107, 1988)

KEY WORDS • parasympathetic and sympathetic nervous systems • aortic diameter • elastic modulus • atropine • propranolol • angiotensin

THE mechanical properties of the walls of large arteries, especially the aorta, play a major role in regulating the interrelations between arterial pressure and dimensions, and hence in determining the degree of cyclic stress of arteries and of baroreceptor stretch inside their walls. If one agrees that the primary function of vascular smooth muscle is to maintain vasomotor tone, then smooth muscle activation by changing vascular tone may have significant effects on the elastic properties of the aorta. Thus, the contribution of autonomic mechanisms to smooth muscle tone should be investigated, since it has been reported that in anesthetized animals, substances that blocked the parasympathetic and sympathetic reflexes augmented the action of many vasoactive drugs. Thus, the purpose of our study was to assess the effects of muscarinic and β-adrenergic blockade by atropine and propranolol on the aorta’s elastic response to short-term infusion of angiotensin by analyzing the instantaneous pressure-dimension relationship in the thoracic descending aorta of conscious dogs.

Materials and Methods

Surgical Procedure

Nine adult, mongrel dogs (males and females) were selected for the study. They were free of systemic infection and parasites and appropriately vaccinated. Anesthesia was induced with intravenous thiopental sodium (20 mg/kg) and, after intubation, maintained with enflurane (Abbott) 2.5% delivered through a Bain tube connected to a Bird Mark 8 ventilator. A left lateral thoracotomy was made at the level of the fourth intercostal space. A Konigsberg P7 miniature pressure gauge, to which was attached a K30 catheter for in situ calibration, was passed through the left brachial artery so that the pressure sensor lay in the descending aortic lumen 2 cm distal to the origin of the left brachial artery. The fatty tissue covering the upper third of the descending aorta was dissected, and two 5-MHz piezoelectric crystals of 4 mm diameter were diametrically opposed in the adventitia. Final localization was achieved by monitoring the output of a sonomi-
crometer (Triton Technology Inc.) to find the place of largest diameter. A catheter was then placed in the left internal mammary vein for subsequent infusion of drugs. All cables and catheters were tunneled subcutaneously to emerge at the midscapular region of the back. The thoracotomy was closed and the animal allowed to recover under veterinary supervision, which included routine antibiotic treatment.

**Measurements**

Aortic blood pressure was measured with the implanted pressure gauges, which were calibrated in vitro before surgery and in vivo by reference to a P23 transducer (Statham Instruments, Hato Rey, Puerto Rico) connected to the aortic catheter.\(^5\)\(^6\) The transducer was calibrated using a mercury column. When the signal had been calibrated, the transducer was disconnected from the aortic catheter, which was then closed. The aortic pressure signal was displayed on the oscilloscope. External aortic diameter was measured by an ultrasonic dimension technique that determines the transit time of acoustic impulses traveling at a velocity of approximately \(1.5 \times 10^6\) mm/sec between the pairs of implanted piezoelectric crystals.\(^5\)\(^6\) The cables attached to piezoelectric crystals were connected to the sonomicrometer and the output signal was monitored on an oscilloscope. The aortic diameter and pressure signals were recorded on a FM tape recorder (Model 3968A, Hewlett-Packard, Palo Alto, CA, USA) and analyzed in terms of mean values and systolic-diastolic variations. This enabled calculation of the pressure elastic modulus (EP) proposed by Peterson et al.\(^7\) for arteries in vivo according to the following formula: \(\text{EP} = \Delta P \times D/\Delta D\), where \(\Delta P\) and \(\Delta D\) are the systolic-diastolic changes in pressure and diameter, respectively, and \(D\) is the mean diameter calculated by electronic integration of the instantaneous diameter curve. Assuming an isotropic homogeneous elastic medium for the aortic wall, the incremental elastic modulus (EI) was given by the following formula: \(\text{EI} = (0.75 \times \text{EP})/(hK^2)\), where \(K\) is the external radius and \(h\) the wall thickness. The in vivo external radius was measured as described above. Wall thickness was estimated by counting lamellae on our histological preparations according to Wolinsky’s methodology proposed for interpreting physiological data.\(^8\)

**Pharmacological Protocol**

Pharmacological experiments were performed when the animals had recovered from surgery, 8 days postoperatively. All animals were active, eating well, and free of any apparent signs of infection. They were conscious and unsedated. The investigations were performed with the animals lying quietly on one side. A 5% dextrose drip infusion was started through the mammary vein catheter for administration of drugs. For autonomic blockade,\(^9\) atropine (0.2 mg/kg), propranolol (1.5 mg/kg), and atropine plus propranolol (each at the same doses) were administered separately at 2-day intervals and in random order for each animal. Among the nine dogs studied, the various autonomic blockade treatments were randomized in the same dog so that four groups of five dogs were obtained as follows: 1) dogs receiving only propranolol, 2) dogs receiving atropine alone, 3) dogs receiving propranolol alone, and 4) dogs receiving atropine plus propranolol. Acute hypertension was induced by intravenous infusion of angiotensin delivered in six incremental steps of 0.63, 1.25, 2.05, 5, 10, and 20 \(\mu\)g/min. Angiotensin administration was performed once in each dog of each of the four groups. Since several autonomic interventions and their relative angiotensin curves were done in the same dog, each intervention was separated from the previous one by an interval of 2 days in order to obtain complete recovery from the pharmacological maneuvers. Aortic diameter and pressure signals were recorded for each angiotensin dose after achieving a steady state of pressure and diameter of at least 2 minutes’ duration. At the end of the final experiment the animals were killed by an overdose of sodium thiopental, and autopsies were performed to verify correct catheter and crystal placement.

**Statistical Analysis**

Statistical analysis was performed according to standard methods and analysis of variance. Values reported are means ± SEM.\(^10\) Linear correlations were performed by the least-squares method.\(^10\) A p value of less than 0.05 was considered statistically significant.

**Results**

**Before Angiotensin Administration**

Table 1 shows the baseline values of systolic, diastolic, and mean aortic pressures and diameter, as well as heart rate and aortic wall thickness in the absence and presence of atropine and/or propranolol. The age and weight of the dogs in all groups were not significantly different. Compared to the control group, mean aortic pressure and diameter tended to increase after atropine and propranolol, but these changes were not statistically significant. Heart rate was higher in the dogs receiving atropine (\(p < 0.01\)) and atropine plus propranolol (\(p < 0.05\)) than in the control group; it was also higher in the dogs receiving atropine (\(p < 0.05\)) and atropine plus propranolol (\(p < 0.01\)) than in those receiving only propranolol.

**After Angiotensin Administration**

In each group of dogs the variations in aortic diameter (VD) from baseline obtained at the different doses of angiotensin were positively correlated with the corresponding variations in aortic pressure (VP) from baseline, either when the results were expressed in absolute (VD-VP) or percent changes (VD%-VP%). The r values for VD-VP and VD%-VP%, respectively, were as follows: 0.93 and 0.88 in the control group (\(p < 0.001\)); 0.63 and 0.66 in the atropine group (\(p < 0.01\)); 0.97 and 0.96 in the propranolol group (\(p < 0.001\)); and 0.56 and 0.50 in the atropine plus propranolol group (\(p < 0.01\)). Figure 1 shows that the
TABLE 1. Baseline Morphological and Hemodynamic Values Before Angiotensin Administration

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>Atropine group</th>
<th>Propranolol group</th>
<th>Atropine + propranolol group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>35 ± 2</td>
<td>34 ± 2</td>
<td>37 ± 1</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>19 ± 2</td>
<td>21 ± 1</td>
<td>20 ± 1</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>117 ± 3</td>
<td>117 ± 3</td>
<td>130 ± 7</td>
<td>128 ± 9</td>
</tr>
<tr>
<td>Diastolic pressure (mm Hg)</td>
<td>76 ± 4</td>
<td>84 ± 5</td>
<td>84 ± 5</td>
<td>86 ± 4</td>
</tr>
<tr>
<td>Mean pressure (mm Hg)</td>
<td>89 ± 4</td>
<td>95 ± 6</td>
<td>99 ± 5</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>Systolic diameter (mm)</td>
<td>16.2 ± 0.9</td>
<td>17.7 ± 1.1</td>
<td>17.0 ± 0.9</td>
<td>18.0 ± 0.7</td>
</tr>
<tr>
<td>Diastolic diameter (mm)</td>
<td>14.4 ± 0.7</td>
<td>16.6 ± 2</td>
<td>15.4 ± 0.8</td>
<td>16.9 ± 0.8</td>
</tr>
<tr>
<td>Mean diameter (mm)</td>
<td>15.0 ± 0.8</td>
<td>16.9 ± 2</td>
<td>15.9 ± 0.8</td>
<td>17.2 ± 0.8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>114 ± 5</td>
<td>180 ± 10*</td>
<td>114 ± 6</td>
<td>155 ± 10*†</td>
</tr>
<tr>
<td>Wall thickness (mm)</td>
<td>1.69 ± 0.21</td>
<td>1.50 ± 0.18</td>
<td>1.65 ± 0.18</td>
<td>1.57 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± 1 SEM.

*p<0.01, †p<0.05, compared with control group.

†p<0.05, compared with propranolol group.

slopes of the VD-VP and VD%-VP% correlations were lower with atropine (p<0.01) and with atropine plus propranolol (p<0.001) than in the control group, and were lower with atropine (p<0.001) and with atropine plus propranolol (p<0.001) than with propranolol. In contrast, no differences in the slopes existed for any correlation between the propranolol and control groups.

In each group of dogs, the Peterson (EP) and incremental elastic (EI) moduli calculated at the different doses of angiotensin were positively correlated with the corresponding mean aortic pressure (MAP). The r values for EP and EI, respectively, were as follows: 0.66 and 0.79 for controls (p>0.01), 0.53 and 0.52 for atropine (p<0.05), 0.79 and 0.74 for propranolol (p<0.01), and 0.54 and 0.51 for atropine plus propranolol (p<0.05). Figure 1 shows that the slopes of the EI-MAP correlations were higher with
atropine \((p < 0.05)\) and with atropine plus propranolol \((p < 0.05)\) than in the control group, and that the slope of the EP-MAP correlation was higher with atropine plus propranolol \((p < 0.05)\) than in the control group; the slopes of the EP-MAP and EI-MAP correlations were also higher with atropine \((p < 0.05)\) and with atropine plus propranolol \((p < 0.05)\) than with propranolol (see Figure 1). In contrast, no differences in slope existed for any correlation between the propranolol and control groups. Finally, heart rate did not correlate with EP and EI either in controls or in the groups receiving atropine, propranolol, or atropine plus propranolol.

**Discussion**

The present study was conducted to establish whether muscarinic and β-adrenergic blockade by atropine and propranolol can modify in conscious animals the elastic response of the aorta to increased stepwise infusions of angiotensin. Such stepwise infusion was done to achieve a steady pressure-diameter state at each increment of angiotensin, and to determine the capacity of distention of the aorta in response to acute hypertension induced by angiotensin. The aortic distention response did not differ with propranolol and was lower with atropine, suggesting that in response to a given aortic pressure increase, atropine augmented the contractile effect of angiotensin on the aorta of conscious dogs (Figure 2); the fact that the addition of propranolol to atropine did not modify this enhanced contractile effect of angiotensin indicates that the action of atropine was not related to the influence of β-dilator receptors, as previously reported on a canine femoral artery preparation. The reduction in the slope of the pressure-diameter curve by atropine cannot be related to the slight and nonsignificant differences in aortic diameter before angiotensin among control, atropine, and atropine plus propranolol groups: indeed, the slight increase in baseline aortic diameter under atropine was probably due to passive distention of the aorta induced by a small increase in pressure and not to a direct dilator effect of atropine on the aorta. The mechanisms of the decreased capacity of distention of the aorta in response to angiotensin-induced acute hypertension observed after atropine raises the problem of atropine’s effects on the peripheral and central portions of the parasympathetic nervous system. Although the blockade by atropine of neurogenic cholinergic vasodilation of the aortic walls is likely, an effect on the central portion of the parasympathetic nervous system cannot be excluded as a reason for the enhanced responsiveness of aortic contraction to angiotensin.

To confirm the impression obtained from analysis of the pressure-diameter relationship in the aorta, the elastic response of this vessel was analyzed by correlating the elastic moduli (Peterson and incremental values) to the transmural pressure obtained at each stepwise dose of angiotensin. Such moduli constituted a good estimate of dynamic elastic modulus; indeed, it has been shown that the dynamic elastic modulus can be calculated as the product of the incremental elastic modulus (EI) deduced from the pressure-strain modulus (EP) and the cosine of the phase lag of the diameter and pressure corresponding to the same harmonic. Since it was demonstrated by Gow and Taylor that the phase lag between diameter and pressure of the midthoracic aorta of living dogs is small (about 6–12 degrees) the dynamic elastic modulus was approximately identical to the incremental elastic modulus because the cosine of the phase lag is close to 1. The positive correlation between elastic modulus and MAP can be considered as the response of rigidity of the aorta 1) to the greater stretch of its walls because of the uniform composition in elastic materials and 2) to a possible intrinsic contractile effect of angiotensin on the smooth muscle of the aortic wall. However, the slope of the elastic modulus-pressure correlation was clearly increased after atropine blockade, indicating...
that at a given transmural pressure, the aortic stiffness was higher after atropine than before atropine (see Figure 2); the fact that this result was unaffected by propranolol suggests the nonparticipation of the $\beta$-adrenergic receptors of the aorta in this phenomenon. The increased stiffness of the aorta caused by atropine may be due to the enhanced angiotensin-mediated activation of aortic smooth muscle caused by atropine, since the higher the smooth muscle activation of the aorta, the higher the resistance to distention of its walls at any transmural pressure.

Finally, results from the present study indicate that in response to an acute angiotensin-mediated increase in transmural pressure, the distention of the aorta was decreased and the aortic stiffness increased by atropine blockade, independent of the influence of the $\beta$-adrenergic receptors. This is the first time, to our knowledge, that results have been obtained in conscious dogs, and they suggest the possibility of in vivo vasodilating action of the parasympathetic nervous system on the aorta. In addition, the stiffening effect of parasympathetic blockade on the aorta of dogs should be taken into account in interpreting the classic impairment of baroreceptor reflexes by atropine, which may be due in part to a lesser stretch of mechanoreceptors because of the increased rigidity of the arterial wall caused by parasympathetic blockade. However, either central or peripheral (or both) portions of the parasympathetic nervous system could contribute to the observed results. Because atropine affects both the central and peripheral nervous systems, further investigations are required to determine the contribution of both systems by administering, for example, a belladonna alkaloid that does not easily cross the blood-brain barrier.

Acknowledgments

We thank Christine Beretti and Christine Beuzet for their technical and secretarial assistance.

References

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Hypertension. 1988;11:I103
doi: 10.1161/01.HYP.11.2_Pt_2.I103
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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