Losartan Inhibits Sympathetic and Cardiovascular Responses to Carotid Occlusion

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Abstract We have reported that inhibition of angiotensin-converting enzyme with captopril attenuates the cardiovascular responses to bilateral carotid occlusion in conscious rabbits and proposed that the attenuation results from removal of a facilitatory action of angiotensin II on the sympathetic nervous system. The aim of the present study was to assess the effect of carotid occlusion on renal sympathetic nerve activity in conscious rabbits and to investigate the effect of the angiotensin II subtype 1 (AT₁) receptor antagonist losartan on the cardiovascular and renal sympathetic nerve activity responses to carotid occlusion. In seven conscious, aortic depressor nerve-sectioned rabbits, carotid occlusion elicited prompt and reproducible increases in mean arterial pressure from 75±2 to 124±5 mm Hg (P<.001), heart rate from 285±8 to 317±9 beats per minute (P<.01), and renal sympathetic nerve activity to 165±11% of control (P<.01). In the same rabbits, losartan (5 mg/kg IV) decreased mean arterial pressure by 9±2 mm Hg (P<.01), increased renal sympathetic nerve activity to 143±13% of control (P<.05), but did not alter heart rate. Losartan significantly attenuated (P<.01) the mean arterial pressure (66±2 to 81±2 mm Hg), heart rate (282±9 to 289±7 beats per minute), and renal sympathetic nerve activity (143±13% to 159±15% of control) responses to carotid occlusion. The responses to carotid occlusion were not restored when the hypotension produced by losartan was reversed by phenylephrine infusion, and nitroprusside-induced hypotension did not attenuate the pressor or renal sympathetic nerve activity responses to carotid occlusion. These results provide evidence that endogenous angiotensin II facilitates the cardiovascular and sympathetic nerve activity responses to carotid occlusion by an action on central AT₁ receptors.

Key Words • angiotensin II • receptors, angiotensin • losartan • carotid sinus reflex • blood pressure • heart rate • sympathetic nervous system • rabbit

Methods

Experiments were conducted in male New Zealand White rabbits weighing 2.7 to 3.3 kg using procedures approved by the University of California, San Francisco, Committee on Animal Research. The rabbits were housed singly in cages in a room with a constant temperature and a 12-hour light/dark cycle and fed a commercial diet (Purina Rabbit Chow, Ralston-Purina) with water ad libitum.

Surgical Procedures

For surgical preparation, rabbits were anesthetized with a mixture of xylazine (Lloyd Laboratories, 5 mg/kg IM) and ketamine (Parke-Davis, 50 mg/kg IM). Sterile technique was used, and ampicillin (10 mg/d IV) was given postoperatively.

Arterial and Venous Catheters

Two Tygon catheters were inserted into the right external jugular vein. A catheter consisting of silicone elastomer connected to PE-60 tubing was inserted into the right femoral artery. The catheters were led subcutaneously to the back of the neck and protected in the pockets of a nylon mesh jacket. The catheters were flushed with heparinized saline (1000 U/mL) at least every other day.

Aortic Nerve Section and Carotid Occluders

Three days after arterial and venous catheterization, a midline incision was made in the midcervical area to expose both common carotid arteries. The vagosympathetic sheath was incised longitudinally, and the aortic nerve, sympathetic trunk, and vagus were identified. To eliminate the buffering action of the aortic arch baroreceptors and maximize the responses to carotid occlusion, the aortic nerve was sectioned bilaterally. Inflatable balloon vascular occluders (2 mm internal diameter, In Vivo Metric) were placed around both carotid arteries. The incision was closed, and the silicone elastomer tubing from the occluders was exteriorized along with the vascular catheters.
Renal Nerve Electrodes

Seven days after placement of the carotid occluders, electrodes were placed on the left renal nerve for recording of RSNA as described previously.10-13 The left kidney was exposed via a retroperitoneal approach through a left flank incision. With a dissecting microscope, a renal nerve was identified and carefully isolated. Polytetrafluoroethylene-coated multistrand stainless wire electrodes (A-M System, Inc) were placed on the nerve, and a ground lead was fixed to the tissue close to the electrodes. The nerve and recording electrodes assembly was covered in silicone gel (Sil Gel 504, Wacker-Chemie Gimble), and the electrodes were exteriorized at the back of the neck.

Data Acquisition

On the day of an experiment, a rabbit was placed inside the shielded cage, and continuous recordings of arterial pressure, heart rate (HR), and RSNA were begun. Arterial pressure and HR were monitored with a pressure transducer (Cobe Laboratories, Inc) and a custom-built cardiovascular analyzer. RSNA was amplified using a differential amplifier (model AM 502, Tektronix) with a band-pass filter (low, 100 Hz; high, 3 kHz), and the action potentials were filtered, rectified, and integrated using a custom-built envelope detector. Renal neurograms were photographed from an oscilloscope screen (model 7313, Tektronix). Arterial pressure, HR, and integrated RSNA were digitized at 100 Hz, stored, and analyzed with a PDP 11/23+ computer (Digital Equipment Corp) and displayed on a polygraph (Grass Instruments). Background noise was determined when nerve activity was eliminated by an infusion of phenylephrine (40 μg/kg per minute). This value was subtracted from all experimental values of RSNA.

Control values for mean arterial pressure (MAP), HR, and RSNA were taken as the 5-minute average before BCO or drug administration. The initial baseline value of integrated RSNA was defined as 100%. BCO was performed three times in each experimental condition. Each occlusion lasted 1 to 2 minutes, and at least 20 minutes elapsed between them. Data collected during the last minute of each occlusion were averaged and used for subsequent analysis.

Experimental Protocols

Effect of Losartan on the Responses to Carotid Occlusion

The effects of BCO on arterial pressure, HR, and RSNA were tested in seven rabbits. At least 20 minutes after three control responses to BCO had been obtained, the rabbits received a bolus intravenous injection (5 mg/kg) of the AT1 receptor antagonist losartan (Du Pont Merck Pharmaceutical Co). Thirty minutes after the administration of losartan when arterial pressure, HR, and RSNA had stabilized at their new values, the effects of BCO were tested again.

Effect of Phenylephrine on the Responses to Carotid Occlusion

The effects of losartan were examined in three rabbits when its hypotensive action was reversed by administration of phenylephrine. This protocol lasted 5 hours, so losartan was administered as a bolus and infusion. After the control responses to BCO had been obtained, losartan was administered intravenously as a bolus of 5 mg/kg followed by infusion at 1 mg/kg per hour, and after arterial pressure, HR, and RSNA had stabilized at their new values, the effects of BCO were tested again. Losartan was then administered intravenously as a bolus of 5 mg/kg followed by infusion at 1 mg/kg per hour, and after arterial pressure, HR, and RSNA had stabilized at their new values, the effects of BCO were tested again.

Statistical Analysis

Results are expressed as mean±SEM. Comparisons between individual pairs of data were made with the paired t test. Multiple comparisons were made using one-way ANOVA for repeated measures and the Newman-Keuls or Dunnett test. Changes were considered to be statistically significant at a value of P<.05.

Results

Arterial Pressure, Heart Rate, and Renal Sympathetic Nerve Activity Responses to Carotid Occlusion

The effects of BCO on arterial pressure, HR, and RSNA are summarized in Figs 1 through 3. BCO elicited prompt increases in MAP from 75±2 to 124±5 mm Hg (P<.001), HR from 285±8 to 317±9 beats per minute (bpm) (P<.01), and RSNA to 165±11% of
FIG 2. Tracings from a conscious rabbit show heart rate (HR), renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP), and pulsatile arterial pressure (AP) responses to bilateral carotid occlusion under control conditions and after administration of losartan. bpm indicates beats per minute.

control ($P<.01$). Reproducible responses were obtained when BCO was repeated 10 times over a 5-hour period.

Effect of Losartan on the Responses to Carotid Occlusion

Losartan decreased MAP from 75±2 to 66±2 mm Hg ($P<.01$), increased RSNA to 143±13% of control ($P<.05$), but did not change HR (286±8 to 282±9 bpm, $P=.40$) (Figs 2 and 3). Losartan markedly attenuated ($P<.01$) the MAP (66±2 to 81±2 mm Hg), HR (282±9 to 289±7 bpm), and RSNA (143±13% to 159±15% of control) responses to BCO (Figs 2 and 3).

Effect of Phenylephrine on the Responses to Carotid Occlusion

Under control conditions, BCO increased MAP from 79±9 to 136±9 mm Hg ($P<.01$), HR from 291±9 to 317±8 bpm ($P<.05$), and RSNA to 167±11% of control ($P<.05$) (Fig 4). Losartan again decreased MAP from 79±9 to 73±4 mm Hg ($P<.05$), increased RSNA to 160±11% of control ($P<.01$), and attenuated ($P<.05$) the MAP, HR, and RSNA responses to BCO. When an infusion of phenylephrine was superimposed on the losartan infusion, MAP increased from 73±5 to 85±4 mm Hg ($P<.05$), HR tended to decrease from 304±16 to 278±21 bpm ($P=.07$), and RSNA decreased from 160±11% to 84±11% of control ($P<.01$). However, phenylephrine did not restore the MAP, HR, or RSNA responses to BCO (Fig 4).

Effect of Nitroprusside on the Responses to Carotid Occlusion

Under control conditions, BCO increased ($P<.05$) MAP from 75±7 to 122±5 mm Hg, HR from 313±22 to 347±19 bpm, and RSNA to 132±7% of control (Fig 5). Infusion of nitroprusside decreased MAP from 75±7 to 67±5 mm Hg ($P<.05$) and tended to increase HR and RSNA. In contrast to losartan, however, nitroprusside did not significantly alter the MAP (67±5 to 115±6 mm Hg) or RSNA (139±31% to 163±27% of control) response to BCO but tended to decrease the HR (330±9 to 350±9 bpm) response. When losartan was superimposed on the nitroprusside infusion, MAP, HR, and RSNA did not change significantly. However, under these conditions losartan again attenuated ($P<.05$) the MAP, HR, and RSNA responses to BCO (Fig 5).
Discussion

In a previous study we demonstrated that inhibition of the renin-angiotensin system with the angiotensin-converting enzyme inhibitor captopril markedly attenuates the cardiovascular responses to BCO. This attenuation could be reversed by Ang II replacement, suggesting that it resulted from a reduction in endogenous Ang II levels. However, a role of increased levels of kinins or vasodilator prostaglandins could not be completely ruled out. The present finding that blockade of AT1 receptors by losartan also attenuates the cardiovascular responses to BCO argues against a role of kinins or vasodilator prostaglandins and provides evidence that AT1 receptors play an important physiological role in facilitating the cardiovascular responses to BCO.

In our previous study we proposed that the attenuation of the cardiovascular responses to BCO resulted at least in part from removal of a presynaptic action of Ang II to facilitate the sympathetic response to BCO. Other studies also support the concept that Ang II facilitates sympathetic activity by a presynaptic action.
Although this action could be exerted at various sites, including sympathetic nerve endings, autonomic ganglia, or the central nervous system, the site and mechanism by which inhibition of the renin-angiotensin system attenuates the cardiovascular responses to BCO in our previous study were not identified. The present finding that losartan attenuated the RSNA response to BCO suggests that this facilitation results from a central action of Ang II. Furthermore, the attenuation of cardiovascular responses to BCO by inhibition of the renin-angiotensin system can be accounted for on the basis of inhibition of sympathetic nerve response to BCO. It should be considered that the attenuation of the RSNA response to BCO by losartan was due to its hypotensive action. However, restoration of RSNA to the prelosartan levels by administration of phenylephrine did not restore the RSNA response to BCO. We also demonstrated that a reduction in arterial pressure by nitroprusside did not attenuate the pressor or RSNA responses to BCO. It is therefore unlikely that the attenuation of the RSNA response to BCO by losartan was due to its hypotensive action.

In conclusion, the present study demonstrates that blockade of AT_1 receptors with losartan attenuates the cardiovascular and RSNA responses to BCO in conscious rabbits. These results suggest that endogenous Ang II facilitates the sympathetic response to BCO by an action on central AT_1 receptors and that blockade of this facilitation can account for the attenuation of the cardiovascular responses to BCO by inhibition of the renin-angiotensin system. Thus, the present study provides additional evidence that the Ang II-sympathetic interaction is physiologically important in cardiovascular regulation.

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