Aldosterone Reduces Baroreceptor Discharge in the Dog

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We have previously demonstrated that baroreceptor discharge sensitivity is depressed in dogs with experimental heart failure and that this depressed sensitivity can be reversed by the Na⁺,K⁺-ATPase inhibitor ouabain. This suggests that enhanced Na⁺,K⁺-ATPase activity in baroreceptors is responsible for the blunted baroreceptor discharge sensitivity seen in heart failure state. Because aldosterone, a known stimulator of Na⁺,K⁺-ATPase, is elevated in heart failure the present study was undertaken to determine the effects on baroreceptor discharge of perfusion of the carotid sinus with aldosterone in normotensive dogs. Single unit baroreceptor activity was recorded as well as carotid sinus pressure and the diameter of the carotid sinus. Perfusion of the carotid sinus with aldosterone (in Krebs-Henseleit solution) significantly elevated threshold pressure (108.5±3.1 mm Hg versus 92.7±4.6 mm Hg, p<0.05) and reduced peak discharge rate (40.3±3.9 spikes/sec versus 47.9±3.6 spikes/sec, p<0.05). These effects appeared 15 minutes after aldosterone perfusion and remained constant for the next 60 minutes. There was no change in the carotid sinus pressure–diameter curve during perfusion with aldosterone. Perfusion of the carotid sinus with ouabain (0.1 μg/ml) during aldosterone perfusion did not reverse the blunted baroreceptor discharge. The blunted baroreceptor activity induced by perfusion of the carotid sinus with aldosterone was prevented by removal of the endothelial cells in the carotid sinus area with a balloon-tipped catheter or by perfusion with saponin. Finally, perfusion of the carotid sinus with spironolactone (10 ng/ml), a mineralocorticoid receptor antagonist, prevented the inhibitory effect of aldosterone. These data suggest that aldosterone reduces maximum baroreceptor discharge. The mechanism for this effect does not appear to involve Na⁺,K⁺-ATPase. It is, however, related to some aspect of carotid sinus endothelial cell function. (Hypertension 1992;19:270–277)

KEY WORDS • aldosterone • carotid sinus • baroreceptors • ouabain • sodium-potassium ATPase • endothelium • saponins • spironolactone • dog studies

In previous studies we demonstrated that carotid sinus baroreceptor sensitivity is depressed in dogs with high output1-2 or low output3-4 heart failure. The depressed baroreceptor sensitivity in low output heart failure can be partially reversed by perfusion of the carotid sinus with low doses of the cardiac glycoside ouabain.3-4 These data suggest that increased Na⁺,K⁺-ATPase activity is responsible for the depressed baroreceptor sensitivity in the dogs with heart failure. The mechanism that is responsible for the apparent increase in Na⁺,K⁺-ATPase activity in these nerve endings is not known. In many forms of severe heart failure there is a significant stimulation of the renin-angiotensin-aldosterone system.5-11 It is well accepted that aldosterone is a potent stimulator of the sodium pump in many tissues.12-19 It is possible that elevated aldosterone may be responsible for the depressed baroreceptor sensitivity in the heart failure state. On the other hand, peripheral or central administration of aldosterone can induce hyper-tension.20-25 This effect may be mediated by a depressed baroreceptor reflex. In the present experiments, we hypothesized that aldosterone can lead to an inhibition of the baroreceptor reflex by stimulation of baroreceptor Na⁺,K⁺-ATPase.

The purpose of the present study was to determine the effects of aldosterone on baroreceptor activity in normal anesthetized dogs in which the carotid sinus was isolated and perfused with a control solution or a solution containing levels of aldosterone that are found in the heart failure state. The results obtained indicate that local perfusion of the carotid sinus with aldosterone significantly blunted baroreceptor activity without a change in the vessel compliance. This blunted baroreceptor activity was not reversed by the Na⁺,K⁺-ATPase inhibitor ouabain but can be prevented by removal of endothelial cells or by the aldosterone antagonist spironolactone.

Methods

Thirty-three normotensive adult mongrel dogs of either sex, weighing from 16 to 35 kg (average 23.3±0.9 kg) were used in the present study. Each dog was anesthetized with sodium pentobarbital (30 mg/kg i.v.) and intubated. A femoral artery was catheterized for acquisition of blood samples and for systolic, diastolic, pulse, and mean arterial pressure measurements. A femoral vein was cannulated for administration of supplemental doses of pentobarbital. Arterial blood gases...
Preparation of Isolated Carotid Sinus

Through a midline incision in the neck, the left carotid sinus area was exposed. The common carotid, external carotid, and lingual arteries were catheterized. All other branches of the carotid sinus region were ligated. Carotid sinus pressure (CSP) was measured using a Millar transducer-tipped catheter (model PC-350, Millar Instruments, Inc., Houston, Tex.) inserted into the external carotid artery, the tip of which was located at the bifurcation of the common carotid artery into the internal and external carotid arteries. The inflow and outflow perfusion catheters were placed in the common carotid artery and the lingual artery, respectively. The sinus was perfused from a reservoir with an oxygenated Krebs-Henseleit solution at 38°C and pH 7.4 containing (in mM) NaCl 129, KCl 4.8, CaCl₂ 1.1, MgSO₄ 2.5, KH₂PO₄ 1.2, NaHCO₃ 25, dextrose 5.5, pyruvate 2.0. The CSP was controlled by adjusting a regulator valve connected to a pressurized air source that pressurized the reservoir and by adjusting the outflow resistance distal to the pressure catheter with an adjustable tubing clamp. The carotid sinus perfusion rate was kept at approximately 10 ml/min. All innervation to the carotid sinus was sectioned except for the carotid sinus nerve.

Single Unit Baroreceptor Recordings

The carotid sinus nerve was cut at its junction with the glossopharyngeal nerve trunk. The nerve was immersed in a warm mineral oil bath, placed on a small mirror, and desheathed. Fibers were continuously split and placed on a platinum-iridium unipolar electrode until activity from a single carotid sinus baroreceptor was recorded. Single fibers were verified by uniform spike amplitude and relatively constant interspike intervals at a suprathreshold static pressure. The single unit discharge activity was amplified with a Grass D.C. preamplifier (model P18D, Grass Instruments Co., Quincy, Mass.) with the low-frequency cutoff set at 3 kHz. The amplified discharge was monitored on a storage oscilloscope (model 121N, Tektronix, Beaverton, Ore.) and was connected to a neuronal spike analyzer (model N750, Mentor, Minneapolis, Minn.). A window discriminator was set so that impulses from only one fiber were discriminated even if activity from more than one fiber was recorded. The discriminator pulses thus corresponded only to the desired single unit baroreceptor discharge. The raw nerve activity, discriminator pulses, and carotid sinus pressure were all recorded on an FM tape recorder (model D, Vetter, Rebersberg, Pa.) and on an electrostatic strip chart recorder (model ES 1000B, Gould Inc., Glen Burnie, Md.).

Carotid Sinus Diameter Measurement

A pair of 2 mm piezoelectric crystals (5 MHz) were placed on the medial and lateral aspects of the carotid bifurcation. The crystals were secured with one 5-0 suture placed through the adventitia. The diameter was measured with a sonomicrometer dimension system (Triton Technology, Inc., San Diego, Calif.).

Removal of Endothelial Cells

Two techniques were used to remove endothelial cells. In three dogs, a mechanical removal technique was used. A Fogarty embolectomy catheter (4F) was inserted into the isolated carotid sinus. The balloon was then inflated and slowly withdrawn. This procedure was repeated three or four times. In two experiments, balloon rubbing depressed receptor discharge temporarily. The CSP-discharge curve was completely normal, however, approximately 30 minutes after denudation. A chemical removal technique was used in another five dogs. This entailed perfusion of the carotid sinus with 0.5 mg/ml of saponin (Fisher Scientific, Fairlawn, N.J.) in oxygenated Krebs-Henseleit solution for approximately 5 minutes. Successful denudation was confirmed by scanning and transmission electron microscopy in three experiments and by elimination of acetylcholine-induced (10⁻⁴ M) vasodilatation of the carotid sinus in all experiments. There was no difference in the data obtained by either method of endothelial cell denudation. Therefore, the data were pooled for statistical purposes.

Experimental Protocol

After a single baroreceptor fiber was isolated, CSP was kept at 100 mm Hg with static pressure for at least 20 minutes. Then the CSP was rapidly reduced to zero and the sinus was exposed to a slow ramp increase in CSP (5 mm Hg/sec) from zero to threshold pressure (i.e., the pressure at which activity was initiated). From the threshold pressure, CSP was increased stepwise; each step was about 25 mm Hg and lasted 10–15 seconds. Pressure was increased up to 250 mm Hg. Thereafter, a 1.5 Hz (90/min) sinusoidal oscillation was added to the mean perfusion pressure using a multifunction pressure generator (model WGA-200, Millar Instruments, Inc., Houston, Tex.). Pulse pressure was adjusted to be between 40 and 50 mm Hg and was held constant within a given experiment. A second curve was then constructed by increasing the mean CSP as described above. A CSP–diameter curve was generated using the same techniques. After control curves were constructed, the sinus was perfused with d-aldosterone (Sigma Chemical Co., St. Louis, Mo.) at a dose of 50–500 pg/ml in Krebs-Henseleit solution at a static pressure of 100 mm Hg. Fifteen minutes later, both static and pulsatile CSP–discharge and CSP–diameter curves were regenerated. Similar curves were constructed every 15 minutes for the next 45 minutes. In a second series of eight dogs, ouabain (0.1 μg/ml) was added to the perfusion solution 15 minutes after beginning perfusion of the carotid sinus with aldosterone. For these experiments, three CSP–discharge curves were constructed (control, 15 minutes after beginning perfusion of aldosterone, and 15 minutes after ouabain plus aldosterone). In three baroreceptors recorded from two dogs, a dose–response relation for aldosterone was examined. The concentrations of aldosterone used were 50, 100, and 500 pg/ml. The reversibility of the aldosterone effect was examined in these units after reperfusion with normal Krebs-Henseleit solution for 60 minutes. In a third series of eight dogs, aldosterone was
administered after removal of the carotid sinus endothelium with a balloon-tipped catheter (n=3) or with saponin (n=5). The effect of aldosterone on baroreceptor activity after endothelial denudation was again studied. We waited 15–30 minutes after denudation to examine the baroreceptor response to aldosterone. At the conclusion of three of these experiments, the carotid sinus was excised and stretched to its in vivo length (at 100 mm Hg) and fixed in 2% glutaraldehyde for scanning and transmission electron microscopy. In the last series of five dogs, aldosterone was infused after prior perfusion with 10 ng/ml of spironolactone for 20 minutes to determine if the effect of aldosterone is receptor specific.

Data Analysis

The CSP, single unit discharge rate, and carotid diameter were sampled during the last 5 seconds of each pressure step with a Tecmar A/D (Tecmar, Inc., Solon, Ohio) converter in an IBM XT computer. CSP–discharge and CSP–diameter curves were constructed from these data. These data were fit with a second order polynomial equation. The first derivative of the equation for CSP–discharge data was determined to derive the slope of the pressure–discharge relation. The threshold and the gain (the slope of the pressure–discharge curve) were determined for each curve generated on each fiber. The data from all fibers are expressed as mean±SEM. A two-way analysis of variance followed by the Newman–Keuls test was used to determine significant differences between control, aldosterone, and aldosterone plus ouabain curves; predenudation and postdenudation; or postdenudation and aldosterone curves. A similar analysis was done to determine significant differences between control, aldosterone, and aldosterone plus spironolactone curves as well as for comparisons of preendothelial and postendothelial denudation experiments. Differences with a statistical probability of less than 0.05 were considered significant.

Results

Effects of Aldosterone on Carotid Sinus Baroreceptor Activity

Figure 1 shows an original recording of the effects of local perfusion of the carotid sinus with aldosterone on single unit baroreceptor discharge in an individual dog. The recording was made using pulsatile pressure. As can be seen, 15 minutes after aldosterone perfusion at a dose of 500 pg/ml, the discharge rate for this unit has been reduced during increases in CSP. Figures 2 and 3 summarize the effects of aldosterone on baroreceptor discharge in response to static (n=17 fibers) and pulsatile (n=11 fibers) pressure. The threshold pressure was significantly increased after aldosterone from 92.7±4.7 mm Hg to 108.5±3.1 mm Hg (p<0.05) during static pressure steps and from 69.3±5.0 mm Hg to 88.8±3.3 mm Hg (p<0.05) during pulsatile pressure steps. The peak discharge rate was significantly decreased after aldosterone perfusion from 47.9±3.6 spikes/sec to 40.3±3.9 spikes/sec (p<0.05) during static pressure steps and from 60.4±6.3 spikes/sec to 47.3±4.9 spikes/sec (p<0.05) during pulsatile pressure steps. There was very little effect of aldosterone on the slopes of the pressure–discharge curves except during pulsatile steps. The slopes were slightly but significantly reduced at pressures above 150 mm Hg during aldosterone. There was no effect of perfusion with normal Krebs-Henseleit solution for 60 minutes on baroreceptor discharge or threshold pressure.

In three fibers recorded in two additional dogs the effects of aldosterone on receptor discharge were evaluated at lower doses. As can be seen in Figure 4, a dose-dependent reduction in baroreceptor discharge at high pressures was seen. In addition, washing out the carotid sinus with normal Krebs-Henseleit solution for 60 minutes reversed the depression exhibited by aldosterone.

Effects of Aldosterone on Carotid Sinus Compliance

In eight dogs, the effects of local carotid sinus perfusion with aldosterone on carotid sinus compliance were
investigated. As can be seen in Figure 5, there was no change in the CSP-diameter curves during perfusion with aldosterone compared with the control perfusion for both static and pulsatile pressures.

**Influence of Ouabain on the Effects of Aldosterone on Baroreceptor Activity**

In another eight dogs, aldosterone was perfused after a control CSP-discharge curve was constructed. When the threshold was significantly elevated and the peak discharge rate was blunted, the Na⁺,K⁺-ATPase inhibitor ouabain (0.1 μg/ml) was added to the perfusion solution in the presence of aldosterone. Ouabain had no effect on the depression of the CSP-discharge curve evoked by aldosterone (Figure 6). This suggests, therefore, that the mechanism by which aldosterone inhibits peak discharge and increases threshold is not related to its effects on Na⁺,K⁺-ATPase.  

**Influence of Denudation of the Carotid Sinus Endothelium on the Effects of Aldosterone on Baroreceptor Discharge**

Both balloon rubbing and saponin perfusion denuded the carotid sinus endothelium. As is shown in Figure 7 the acetylcholine-induced vasodilation ($1 \times 10^{-6} \text{ M}$) was completely abolished after denudation; however, the nitroglycerin-induced vasodilation ($3.5 \times 10^{-6} \text{ M}$) re-

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**FIGURE 2.** Line plots show effects of aldosterone (500 pg/ml) on the carotid sinus pressure (CSP)-discharge curve (upper panels) and its slope (lower panels) in response to static (left panels, n=17) and pulsatile (right panels, n=11) pressure. *p<0.05. Aldo, aldosterone.

**FIGURE 3.** Bar graphs show mean data for threshold pressure and peak discharge rate for curves generated with static (n=17) and pulsatile (n=11) pressure in the control state and during perfusion with 500 pg/ml aldosterone. A significant increase in threshold and decrease in peak discharge rate was noted during aldosterone perfusion. *p<0.05. Aldo, aldosterone; P, pressure; sp, spikes.

**FIGURE 4.** Line plot shows dose-response relations for intracarotid aldosterone. Data are the mean of three fibers in two dogs. These curves were derived from data obtained using static pressure steps. Aldo, aldosterone; CSP, carotid sinus pressure.
In addition, electron microscopy confirmed endothelium denudation. Figure 8 shows transmission electron micrographs of intact endothelium (Figure 8A) and denuded endothelium (Figure 8B) after perfusion with saponin.

Saponin and balloon denudation had no sustained effects on baseline baroreceptor discharge or on the CSP–discharge curve. However, as is shown in Figure 9, the blunted baroreceptor discharge induced by aldosterone was prevented after removal of the endothelium.

Influence of Spironolactone on the Effects of Aldosterone on Baroreceptor Discharge

To determine the specificity of the aldosterone effect on baroreceptor discharge, we perfused aldosterone after prior perfusion of the carotid sinus with 10 ng/ml of spironolactone for 20 minutes in five dogs. As can be seen in Figure 10, the aldosterone-induced baroreceptor inhibition was completely prevented by spironolactone.

Discussion

It has been shown that both carotid and aortic baroreceptor sensitivity is significantly depressed in experimental low and high output heart failure.\(^1,4,30-33\) The depressed sensitivity of carotid sinus baroreceptors in low output heart failure can be partially reversed by local carotid sinus perfusion with a relatively low dose of a cardiac glycoside.\(^3,4\) These data suggest that an enhanced Na\(^+\),K\(^+\)-ATPase activity in the carotid sinus baroreceptor membrane is responsible for the depressed sensitivity observed in the heart failure state.

Several abnormalities of neurohumoral function have been well documented in heart failure. These include elevations in catecholamines, atrial natriuretic peptide, and the renin-angiotensin-aldosterone system. It is well accepted that aldosterone can stimulate Na\(^+\),K\(^+\)-ATPase in the renal cortical collecting tubule.\(^12-19\) In addition, chronic administration of aldosterone or deoxycorticosterone acetate can induce hypertension. Both central and peripheral administration of aldosterone\(^20-25\) can induce hypertension in animals on both normal and high salt intake. It is unclear whether any of the aldosterone-induced hypertension is mediated by effects on the baroreceptor...
reflex, either centrally or at the receptors themselves. It is possible that aldosterone may depress baroreceptor activity by increasing Na⁺,K⁺-ATPase in the carotid sinus. In the present study, perfusion of the carotid sinus with aldosterone significantly increased the threshold and decreased the peak discharge rate of single baroreceptor units in the carotid sinus nerve (Figures 1–3) in a dose-dependent manner (Figure 4). Although we could reverse the effects of aldosterone by perfusing with normal Krebs-Henseleit solution, reversal was seen only after a period of approximately 1 hour. This suggests that aldosterone is tightly bound to its membrane or cytosolic receptor site. This effect on baroreceptor discharge can be prevented by the aldosterone receptor antagonist spironolactone (Figure 10), indicating the involvement of a specific mineralocorticoid receptor. Since there was no change in the carotid sinus–diameter relation during aldoste-
rone (Figure 5), the effects of aldosterone on baroreceptor discharge cannot be explained by a change in the compliance of the carotid sinus.

In a recent study, Moura and Worsel reported that aldosterone had a direct stimulatory action on both ouabain-independent and ouabain-dependent sodium efflux in vascular smooth muscle. There was a very rapid increase in the ouabain-insensitive $\text{Na}^+$ efflux in the rat tail artery. This phenomenon began as early as 15 minutes after aldosterone had been added. This was followed by a secondary rise in the passive efflux of $\text{Na}^+$ that reached a plateau in about 4 hours. This latter phenomenon was blocked by ouabain or prevented by actinomycin D. This latter phenomenon was blocked by ouabain or prevented by actinomycin D. These data suggest, therefore, that both ouabain-sensitive and ouabain-insensitive aldosterone effects operate in the control of $\text{Na}^+$ transport in vascular smooth muscle. In the present experiments, the depressed baroreceptor discharge induced by aldosterone appeared within 15 minutes and could not be reversed by ouabain (Figure 6). This clearly indicates that the baroreceptor depression seen in these experiments is not mediated by an effect on $\text{Na}^+,K^+$-ATPase.

In those situations in which aldosterone would be increased systemically, such as in severe congestive heart failure or in primary hyperaldosteronism, there may be several loci of action in its ability to alter baroreceptor reflex function. It could act centrally, such as in the nucleus tractus solitarius, to effect global baroreceptor reflex function, or it could act peripherally on the afferent baroreceptors or efferent sympathetic nerve endings. In addition, aldosterone could act on the baroreceptor cell bodies in the petrosal ganglion. All could contribute to the generation of transient and possibly chronic hypertension. In a recent study by Janiak et al., it was clearly demonstrated that the reflex bradycardia induced by intravenous phylephenrine was attenuated in deoxycorticosterone acetate-salt hypertensive rats. This apparent inhibition of the baroreceptor reflex could be inhibited by the central or peripheral administration of a specific mineralocorticoid antagonist, RU 28318. These data strongly suggest that not only can mineralocorticoids enter the brain and bind to receptors in the central nervous system, but they may have peripheral mechanisms of action to attenuate baroreceptor reflex function as well. The results of the present study add an additional dimension to the action of mineralocorticoids on the baroreceptor reflex, that is, a direct effect in the carotid sinus.

In the preparation used in the present experiments, the carotid sinus was isolated and perfused, and the carotid sinus nerve was cut. Therefore, the effects of aldosterone must be on the baroreceptor membrane itself or on other structures within the carotid sinus, such as on the endothelial cell or smooth muscle.

Recently, several substances released by endothelial cells have been shown to modulate baroreceptor activity. It is possible that the effects of local, acute perfusion of the carotid sinus with aldosterone on baroreceptor activity are mediated by the carotid sinus endothelium. Since, in the present study, the blunted baroreceptor discharge evoked by aldosterone was abolished after removal of the carotid sinus endothelial cells (Figure 9), we suggest that the effect of aldosterone is endothelium-dependent. In the present experiment, we used two methods to remove the carotid sinus endothelial cells. Both functional (Figure 7) and morphological (Figure 8) data confirmed that the denudation was successful. Furthermore, neither balloon rubbing nor saponin perfusion had any effect on the carotid sinus pressure–discharge curve. This implies that there is no tonic effect of the endothelium on baroreceptor activity. Recently, Chen et al. reported that removal of endothelium with a balloon catheter or a jet of 95% $\text{O}_2$–5% $\text{CO}_2$ gas mixture significantly decreased the slope of the CSP-activity relation in the rabbit. We also found a temporary inhibition of baroreceptor activity after denudation of the endothelium with balloon rubbing that recovered in about 30 minutes. We found no change in baroreceptor activity after denudation with saponin, however. The difference between the study of Chen et al. and our data is not clear. It may be related to the difference in species or to the technique of endothelial cell denudation.

Although there was no reduction in the slope of the pressure–discharge curve in this study (except at high CSP for pulsatile curves), the fact that the peak discharge was reduced and the threshold was increased could indicate that aldosterone contributes to increases in sympathetic outflow or to lack of sympathoinhibition at high pressures. Does this phenomenon have relevance to the heart failure state? As indicated above, it is possible that hyperaldosteronism contributes to baroreceptor reflex abnormalities differently on a chronic basis than it does acutely. Even though CSP is unlikely to reach high levels in severe heart failure, the increase in baroreceptor threshold pressure could contribute to increases in sympathetic outflow in heart failure.

In summary, local perfusion of the carotid sinus with aldosterone significantly suppressed maximal baroreceptor activity without a change in the vessel compliance. This blunted baroreceptor activity cannot be reversed by the $\text{Na}^+,K^+$-ATPase inhibitor ouabain, but it can be prevented by removal of endothelial cells or by the aldosterone receptor blocker spironolactone. These data suggest that aldosterone reduces baroreceptor discharge, especially in the hypertensive range. Although it is not clear if aldosterone participates in the depression of baroreceptor discharge in heart failure, sustained hyperaldosteronemia may contribute to the depression of baroreceptor reflex function through an endothelial cell mechanism. Further experiments are necessary to determine the effects of aldosterone on other components of the baroreceptor reflex arc.

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