Chronic Administration of Aldosterone Depresses Baroreceptor Reflex Function in the Dog

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Abstract. In a previous study it was shown that acute perfusion of aldosterone into the isolated carotid sinus decreased baroreceptor activity. The aim of the present study was to determine whether chronic, systemic administration of aldosterone also depresses baroreflex function. In six conscious dogs, the baroreflex was determined before and 10 days after an osmotic minipump containing aldosterone (100 μg/kg in 2 mL) was implanted. The slope of the relation between systolic arterial pressure and heart rate was significantly blunted after aldosterone administration (9.1±0.7 versus 13.3±1.2 for nitroglycerin, P<.01; 23.4±5.0 versus 40.1±5.0 for phenylephrine, P<.01). Baroreflex slopes did not change in a sham group (minipump with saline) and an aldosterone plus spironolactone (600 mg/d) group. Plasma aldosterone levels were significantly elevated after the aldosterone minipump was implanted (443±72 versus 37±11 pg/mL, P<.001). Mean arterial pressure was not significantly increased after aldosterone (106.5±3.8 versus 100.4±2.6 mm Hg, P=.2). On the 10th day after aldosterone or saline infusion, an acute experiment was carried out. Single baroreceptor fibers were recorded from the carotid sinus nerve. Compared with the sham group, the threshold was significantly elevated in the aldosterone group (111.3±2.1 versus 85.8±2.8 mm Hg), and the peak discharge rate was markedly decreased (32.5±1.5 versus 54.7±2.5 spikes per second, P<.01). The depressed baroreceptor function could be partially restored after a bolus injection of the Na⁺,K⁺-ATPase inhibitor ouabain (5 μg/kg IV). These data indicate that chronic administration of aldosterone decreases baroreflex function without inducing hypertension. An Na⁺,K⁺-ATPase mechanism is involved in this aldosterone-induced depression of baroreceptor function. This mechanism may be involved in the blunting of the baroreflex in heart failure and other hyperaldosteronemic states. (Hypertension. 1994;24:571-575.)

Key Words • aldosterone • carotid sinus • ouabain • pressoreceptors • Na⁺,K⁺-transporting ATPase • dogs • spironolactone

I t has been demonstrated by this laboratory that baroreceptor discharge sensitivity is depressed in dogs with experimental heart failure and that this depressed sensitivity can be partially reversed by the Na⁺,K⁺-ATPase inhibitor ouabain.1,2 This suggested that enhanced Na⁺,K⁺-ATPase activity in baroreceptors is partially responsible for the blunted baroreceptor discharge sensitivity seen in heart failure. It is well accepted that aldosterone stimulates an Na⁺,K⁺-ATPase activity in baroreceptors that is partially responsible for the blunted baroreceptor discharge sensitivity seen in heart failure.1,2,3 It has been reported that aldosterone is significantly elevated in heart failure.11-14 In a previous study we determined the effects of acute perfusion (15 minutes) of the isolated carotid sinus with aldosterone on baroreceptor discharge in normal dogs. Administration of aldosterone (500 pg/mL) into the carotid sinus significantly depressed baroreceptor discharge activity. However, this depressed activity by aldosterone was not mediated by an Na⁺,K⁺-ATPase mechanism, as the Na⁺,K⁺-ATPase inhibitor ouabain did not restore this depressed baroreceptor activity. Worcel and Moura16 reported that aldosterone has a direct stimulatory action on both ouabain-independent and ouabain-dependent sodium efflux in vascular smooth muscle. The ouabain-insensitive $^{22}$Na⁺ efflux in the rat tail artery increased very rapidly in their studies. This phenomenon began as early as 15 minutes after aldosterone had been added and was followed by a secondary rise in the passive efflux of Na⁺ that reached a plateau in approximately 4 hours. The latter phenomenon was blocked by ouabain or prevented by actinomycin D. These data suggest that aldosterone has two effects on Na⁺ transport: one is a short-latency and ouabain-independent mechanism, and the other is a long-latency and ouabain-dependent mechanism. Since long-term or acute administration of aldosterone have both central and peripheral effects, the aim of the present study was to determine whether chronic administration of aldosterone depresses baroreceptor and baroreflex functions and whether this depression is mediated by an effect on Na⁺,K⁺-ATPase.

Methods

Chronic Experiment

Twenty-three normal, adult mongrel dogs of either sex weighing 16 to 25 kg were used in the present study. Dogs were divided into three groups: an aldosterone group (n=10), a sham group (n=9), and an aldosterone plus spironolactone group (n=4). All animals were fed and housed according to the institutional guidelines at the University of Nebraska. These studies were approved by the University of Nebraska Medical Center Animal Review Committee. With dogs under sodium pentobarbital anesthesia (30 mg/kg IV), the omocervical artery and jugular vein were catheterized. After dogs had recovered

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from surgery, baseline hemodynamics, baroreflex sensitivity, and plasma aldosterone levels were determined on 2 days with dogs in the conscious state. Thereafter, an osmotic minipump (model 2ML2, Alza Corp) was implanted subcutaneously with dogs under mild sedation (acperomazine, 0.5 mg/kg IM) and local anesthesia. The pump contained aldosterone (100 μg/kg in 2 mL) (aldosterone and aldosterone plus spironolactone groups) or 2 mL saline (sham group). Hemodynamics, baroreflex sensitivity, and plasma aldosterone levels were measured on days 3, 5, 7, and 10 after implantation of the osmotic pump. In the aldosterone plus spironolactone group, spironolactone (600 mg/d) was given orally from day 1 to day 10 after implantation of the osmotic pump.

Arterial baroreflex sensitivity was determined by the slope of the linear regression of pulse interval against the preceding systolic arterial pressure during the responses to a bolus injection of phenylephrine (10 μg/kg IV) and nitroglycerin (25 μg/kg IV). Plasma aldosterone was measured with a radioimmunoassay technique (TKaL-1, Diagnostic Products Corp).

Acute Experiment

On the 10th day after the osmotic pump was implanted, an acute experiment was performed after determination of baroreflex sensitivity. Each dog was anesthetized with sodium pentobarbital (30 mg/kg IV) and intubated. Arterial blood gases were measured throughout the experiment and kept within normal limits (pH 7.35 to 7.45; PCO₂, 30 to 40 mm Hg; PO₂, 85 to 95 mm Hg).

Through a midline incision in the neck, the left carotid sinus area was exposed. The carotid sinus area was reversibly isolated. All branches of the carotid sinus were ligated except for the common carotid and external carotid arteries. Two small hydraulic occluders were placed on the common carotid artery proximal to the thyroid artery and on the external carotid artery, respectively. To measure carotid sinus pressure (CSP) and pressurize the carotid sinus, a catheter was inserted into the thyroid artery with the tip close to the carotid bifurcation. When the occluders were inflated, the carotid sinus was isolated from the circulation, and the carotid sinus could be pressurized through the thyroid arterial catheter, which was connected to a pressure reservoir. All innervation to the carotid sinus was sectioned except for the carotid sinus nerve.

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).

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 DC preamplifier (model P18D, Grass Instrument Co) with the low-frequency cutoff set at 100 Hz and high-frequency cutoff set at 3 kHz. The amplified discharge was monitored on a storage oscilloscope (model 121N, Tektronix) and connected to a neuroanalyst (model N750, Mentor). 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 CSP were all recorded on an electrostatic strip-chart recorder (ES 1000B, Gould Instruments).

After a single baroreceptor fiber was isolated, a CSP discharge curve was constructed. The carotid sinus was exposed to a slow ramp increase in CSP (+3 mm Hg/s) from 25 mm Hg to threshold (ie, the pressure at which activity was initiated) from a reservoir containing oxygenated Krebs-Henseleit solu-

Fig 1. Bar graph shows effect of chronic administration of aldosterone (Aldo.), saline (Sham), and aldosterone plus spironolactone (Spiro.) on plasma aldosterone levels. *P<.05 compared with sham group; **P<.05 compared with day 0.

**Results**

**Chronic Infusion of Aldosterone on Hemodynamics and Baroreflex Function in Conscious Dogs**

Osmotic pump infusion of aldosterone increased plasma aldosterone levels significantly. Fig 1 shows average plasma aldosterone levels in the aldosterone, sham, and aldosterone plus spironolactone groups. Compared with control (day 0, before the osmotic pump was implanted), aldosterone levels in the aldosterone and aldosterone plus spironolactone groups were significantly elevated (443.1±71.7 versus 37.5±10.9 pg/mL, _P_<.001, in the aldosterone group; 731.8±73.0 versus 37.5±10.9 pg/mL, _P_<.001, in the aldosterone plus spironolactone group). There was no change in plasma aldosterone levels in the sham group after the osmotic pump was implanted. There was also no statistical difference in plasma aldosterone levels between the aldosterone and aldosterone plus spironolactone groups. Differences with a statistical probability of less than .05 were considered significant.

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significantly depressed at 10 days in the aldosterone group (9.1±0.7 versus 13.3±1.2 ms/mm Hg, *P<.01*, induced by nitroglycerin; 23.4±4.9 versus 40.1±4.9 ms/mm Hg, *P<.01*, induced by phenylephrine, respectively). There was a significant depression in the phenylephrine slope at 7 days in the aldosterone group. Fig 3 shows the changes in baroreflex sensitivity in each group expressed as a percentage of the baroreflex slope in the control state. There was no significant change in baroreflex sensitivity in the sham and aldosterone plus spironolactone groups.

**Effects of Administration of Aldosterone on Baroreceptor Function in Anesthetized Dogs**

Baseline carotid diameters were compared between the sham and aldosterone groups, and no difference was found (4.23±0.51 versus 4.67±0.62 mm).

**Ouabain Reverses the Depressed Baroreceptor Function Induced by Aldosterone**

The depressed baroreceptor function in the aldosterone group was partially restored after a bolus injection of ouabain (5 μg/kg IV). There was no significant change in baroreceptor function in the sham and aldosterone plus spironolactone groups after ouabain. Fig 5 shows these average data. The pressure threshold was decreased from 106.5±4.0 to 97.0±3.7 mm Hg (*P<.05*), and the peak discharge rate was increased from 35.2±2.5 to 46.0±5.4 spikes per second (*P<.05*) in the aldosterone group after ouabain.

**Discussion**

The major findings in the present study are (1) chronic aldosterone administration depresses baroreceptor and baroreflex functions without inducing hypertension; (2) the aldosterone receptor antagonist spironolactone can prevent this aldosterone-induced baroreceptor and baroreflex depression; and (3) the Na⁺,K⁺-ATPase blocker ouabain can restore depressed baroreceptor function induced by chronic aldosterone administration.

In the present study an osmotic minipump was used for aldosterone administration. The plasma aldosterone levels were around 500 pg/mL after the osmotic pump was implanted, which is close to the levels found in severe heart failure.17-21 This aldosterone level depressed the baroreflex in the conscious state (Fig 3) without inducing hypertension (Fig 2). Since the aldo-

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**Figures**

- **Fig 2.** Line graphs show baseline mean arterial pressure (MAP) and heart rate (HR) in sham, aldosterone (Aldo.), and aldosterone plus spironolactone (Spiro.) groups.
- **Fig 3.** Bar graphs show baroreflex slope during bolus injections of nitroglycerin (NG) (25 μg/kg IV) or phenylephrine (PE) (10 μg/kg IV) in sham, aldosterone (Aldo.), and aldosterone plus spironolactone (Spiro.) groups. *P<.05 compared with day 0.
- **Fig 4.** Graphs show effects of chronic administration of aldosterone (Aldo.) on single baroreceptor function. *P<.05 compared with sham and aldosterone plus spironolactone (Spiro.) (19 fibers from sham group, 45 fibers from aldosterone group, 25 fibers from aldosterone plus spironolactone group). CSP indicates carotid sinus pressure.
- **Fig 5.** Bar graphs show baroreflex slope during bolus injections of nitroglycerin (NG) (25 μg/kg IV) or phenylephrine (PE) (10 μg/kg IV) in sham, aldosterone (Aldo.), and aldosterone plus spironolactone (Spiro.) groups. *P<.05 compared with day 0.
Aldosterone receptor antagonist spironolactone prevented this baroreflex depression, this effect appears to be mediated by an aldosterone receptor. In the present study aldosterone administration had a tendency to increase arterial blood pressure, but this increase was moderate and not significant. In previous studies, chronic aldosterone administration did induce hypertension. Also in previous studies, aldosterone was given centrally via the cerebral ventricles at doses that were ineffective when given systemically. In the present study aldosterone was given systemically for 10 days. It is not clear why aldosterone did not induce hypertension in the present study. It is likely that the ability of aldosterone to induce hypertension is related to continual sodium retention, which most likely did not occur for 10 days in this study. The reason why the plasma aldosterone levels are higher in the aldosterone plus spironolactone group than the aldosterone group (731.7±73 versus 443.1±71.7 pg/mL, P<.05) is not clear. Spironolactone may induce a reduction in aldosterone metabolism by occupying receptors.

Aldosterone is generally thought to exert its major effect in the renal distal tubule and collecting duct. Other sites of action are in the colon, sweat glands, and salivary glands, the inner ear, and the myocardial cells. All these sites are areas of intense electrolyte transport, which may result in fluid reabsorption or secretion. A vast amount of evidence unequivocally shows that aldosterone causes its effects on membrane transport by entering the cell nucleus and initiating a DNA-directed mRNA synthesis in such a fashion that more ATPase is made. The most abundant ATPase in a variety of transporting epithelia is Na⁺,K⁺-ATPase. The effect of aldosterone on sodium transport takes between 30 and 180 minutes to be seen and is long lasting. Data also suggest that aldosterone has a direct effect on cell membranes. Worcel and Moura showed that aldosterone increased sodium efflux in cultured vascular smooth muscle cells. This efflux reached a peak in 15 minutes and was not ouabain sensitive. This confirmed earlier work by these investigators showing a rapid increase in passive sodium efflux that was insensitive to actinomycin D in intact rat tail artery. These data suggest that aldosterone has a membrane effect that is not mediated by Na⁺,K⁺-ATPase. In our previous study, acute administration of aldosterone (15 minutes) caused a depression in carotid sinus baroreceptor function. This depression by acute administration of aldosterone was not mediated by an Na⁺,K⁺-ATPase mechanism because ouabain could not restore this depression. This depression in baroreceptor function by acute aldosterone could be prevented by denudation of the endothelial cells in the carotid sinus area. It was suggested that acute administration of aldosterone might stimulate endothelial cells to release some substance that depresses baroreceptor activity. In the present study chronic administration of aldosterone for 10 days also depressed the carotid sinus baroreceptor activity (Fig 4), and this depression was partially reversed by the Na⁺,K⁺-ATPase blocker ouabain (Fig 5). These data suggest that chronic administration of aldosterone stimulates Na⁺,K⁺-ATPase activity in the carotid sinus baroreceptor and in turn depresses baroreceptor function.

Previous studies from this laboratory have shown that baroreceptor activity is significantly depressed in dogs with experimental heart failure and that this depression can be partially restored by perfusion of the carotid sinus with low doses of the cardiac glycoside ouabain. These data suggest that increased Na⁺,K⁺-ATPase activity is responsible for the depressed baroreceptor sensitivity in dogs with heart failure. It is also well accepted that aldosterone is significantly elevated in heart failure and that aldosterone is a potent ATPase stimulant. In the heart failure state this elevated aldosterone may be responsible for the depressed baroreceptor activity. It should be noted that depressed baroreceptor sensitivity by chronic aldosterone administration (data from Fig 4) can explain the impaired baroreceptor reflex in the conscious state (data from Fig 3), it is not clear whether the effects of aldosterone are completely mediated by...
effects at the baroreceptor ending, in the cell body, or at a central site of action in the case of its effect on the baroreflex.

In summary, chronic aldosterone administration causes an inhibition in baroreceptor and baroreflex functions, and the Na⁺,K⁺-ATPase blocker ouabain partially restores depressed baroreceptor function induced by chronic aldosterone administration. I speculate that this effect of aldosterone on baroreceptors may be one of the mechanisms mediating the depression of baroreceptor and baroreflex functions in heart failure.

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