Aldosterone Nongenomically Worsens Ischemia Via Protein Kinase C-Dependent Pathways in Hypoperfused Canine Hearts

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Abstract—Rapid nongenomic actions of aldosterone independent of mineralocorticoid receptors (MRs) on vascular tone are divergent. Until now, the rapid nongenomic actions of aldosterone on vascular tone of coronary artery and cardiac function in the in vivo ischemic hearts were not still fully estimated. Furthermore, although aldosterone can modulate protein kinase C (PKC) activity, there is no clear consensus whether PKC is involved in the nongenomic actions of aldosterone on the ischemic hearts. In open chest dogs, the selective infusion of aldosterone into the left anterior descending coronary artery (LAD) reduced coronary blood flow (CBF) in the nonischemic hearts in a dose-dependent manner. Also, in the ischemic state that CBF was decreased to 33% of the baseline, the intracoronary administration of aldosterone (0.1 nmol/L) rapidly decreased CBF (37.4 ± 3.8 to 19.3 ± 5.2 mL/min; P < 0.05), along with decreases in fractional shortening (FS) (8.4 ± 0.7 to 5.4 ± 0.4%; P < 0.05) and lactate extraction rate (LER) (−31.7 ± 2.9 to −41.4 ± 3.7%; P < 0.05). The decrease in CBF was reproduced by the infusion of bovine serum albumin-conjugated aldosterone. Notably, these aldosterone-induced deteriorations of myocardial contractile and metabolic functions were blunted by the co-administration of GF109203X, an inhibitor of PKC, but not spironolactone. In addition, aldosterone activated vascular PKC. These results indicate that aldosterone nongenomically induces vasoconstriction via PKC-dependent pathways possibly through membrane receptors, which leads to the worsening of the cardiac contractile and metabolic functions in the ischemic hearts. Elevation of plasma or cardiac aldosterone levels may be deleterious to ischemic heart disease through its nongenomic effects. (Hypertension. 2005;46:113-117.)

Key Words: aldosterone ■ ischemia ■ protein kinases

Aldosterone modulates cardiovascular function in addition to the crucial role in sodium and potassium homeostasis through binding to the intracellular mineralocorticoid receptors (MRs).1 These receptor-mediated effects with transcriptional modulation of target genes are termed genomic effects.2 Recently, another pathway possibly mediated by the specific membrane receptors through which aldosterone acts, ie, nongenomic effects of aldosterone, has been investigated.3–4 Although this nongenomic action of aldosterone was shown in a variety of tissues such as vascular smooth muscle cells or cardiomyocytes, the effects of aldosterone on vascular tone are divergent.5–9 Aldosterone increased systemic vascular resistance in humans, as shown in the first report on the nongenomic effects of aldosterone, whereas aldosterone inhibits vasoconstriction in renal afferent arterioles.7–9 Until now, the rapid nongenomic effects of aldosterone on vascular tone of coronary artery and cardiac functions in vivo under ischemia are not still fully estimated. Moreover, several rapid aldosterone-induced changes of the concentration of intracellular second messengers have been described.6,10,11 Aldosterone is reported to activate protein kinase C (PKC) in distal colon cells and cultured kidney cells and to decrease its activity stimulated by phorbol-12-myristate-13-acetate in rat neonatal cardiomyocytes.12–14 However, there is no clear consensus whether PKC is involved in the nongenomic effects of aldosterone on the vascular tone of coronary arteries in the ischemic hearts. Thus, this study was undertaken to investigate the nongenomic effects of aldosterone on coronary vascular tone and cardiac functions in the in vivo ischemic hearts and a role of PKC in this nongenomic effect in canine hearts.

Materials and Methods

Both aldosterone and spironolactone were purchased from Sigma (St. Louis, Mo), and a specific PKC inhibitor, GF109203X, was
obtained from Calbiochem (La Jolla, Calif). Bovine serum albumin-conjugated aldosterone was purchased from Steraloids Inc. (Newport, RI). The primary antibody and the positive control against min-conjugated aldosterone was purchased from Steraloids Inc.

**Instrumentation**

All procedures were performed in careful conformance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, revised 1996). Experimental protocols were approved by the Osaka University Ethical Committee for Laboratory Animal Use.

Fifty-nine hybrid beagle dogs weighing 14 to 22 kg were anesthetized with pentobarbital sodium (30 mg/kg intravenously). The dogs were prepared as previously described. Briefly, the trachea was intubated and the dog was ventilated with room air mixed with oxygen. The chest was opened through the left fifth intercostal space, and the heart was suspended in a pericardial cradle. After heparinization (500 U/kg), the proximal portion of the left anterior descending coronary artery (LAD) was cannulated and perfused with blood via the carotid artery through an extracorporeal bypass tube. Either coronary perfusion pressure (CPP) or coronary blood flow (CBF) was monitored at this tube. A small collecting tube was inserted into a small coronary vein near the perfused area to sample coronary venous blood. The drained venous blood was collected in a reservoir placed at the level of the left atrium. A pair of ultrasonic crystal probes was placed in the center of the perfused area to allow the measurement of myocardial segment length with an ultrasonic dimension gauge (5 MHz; Schuessler, Cardiff by the Sea, Calif). End-diastolic length was determined at the R wave of the ECG, and end-systolic length was determined at the minimal dP/dt. Fractional shortening (FS) was calculated by the formula [(end-diastolic length−(end-systolic length)]/(end-diastolic length), and served as an index of myocardial contractility of the perfused area.

**Experimental Protocols**

**Protocol I: Effects of an Intracoronary Administration of Aldosterone on Systemic and Coronary Hemodynamics in the Nonischemic Hearts**

First of all, to clarify dose-dependent effects of aldosterone on CBF, 20 dogs were used in this protocol. Vehicle and 3 different doses (0.05, 0.1, and 0.2 nmol/L; n=5 each) of aldosterone were randomly and selectively administered into the LAD through the extracorporeal bypass tube. We continuously infused 60 ng aldosterone in 10 mL saline into the LAD so that the final concentration of this infused aldosterone in coronary circulation became 0.1 nmol/L in the nonischemic hearts for 60 minutes. Hemodynamic parameters including heart rate, CPP, and CBF were measured 5, 10, 20, 30, 45, and 60 minutes after drug infusion.

**Protocol II: Effects of an Intracoronary Administration Aldosterone on Coronary Hemodynamic and Metabolic Parameters in the Ischemic Hearts (Constant Low CPP Model)**

After hemodynamic stabilization, CPP was reduced so that CBF was decreased to 33% of the control CBF using an occluder attached at the extracorporeal bypass tube. After a low level of CPP was obtained, the occluder was manually adjusted to keep CPP constant. All of the hemodynamic parameters were measured 5 minutes after the onset of hypoperfusion. Both coronary arterial and venous blood were sampled for metabolic analysis. Then, we administrated aldosterone (0.1 nmol/L, n=7) into the LAD through the extracorporeal bypass tube. The dose of 0.1 nmol/L of aldosterone was chosen because this dose of aldosterone was the minimal dose to induce the maximal coronary vasoconstriction in protocol I. In other dogs, to test the involvement of PKC in regulating CBF, we infused aldosterone with either a PKC inhibitor, GF109203X (300 ng/kg per minute; n=5), or a MR antagonist spironolactone (10 μg/kg per minute, n=5) in the ischemic hearts. An intracoronary infusion of GF109203X at this dose was reported to inhibit PKC activation without changing the coronary hemodynamic and metabolic parameters.

**Protocol III: Effects of Aldosterone on the Activation of PKC of Coronary Artery With and Without Ischemia**

To check effects of aldosterone on PKC activation in coronary arteries, we used 4 dogs in this protocol. After the 15-minute intracoronary infusion of vehicle or aldosterone (0.1 nmol/L) with and without ischemia, the hearts were excised and the vascular segments from the LAD were modestly separated and quickly placed into liquid nitrogen (LN2) and stored at −80°C. Then, the vascular segments obtained were separated into membrane and cytosolic fractions and the activity of PKC was checked by Western blot analysis as previously described.

**Biochemical Analysis**

Lactate extraction ratio (LER) was calculated by multiplying the coronary arteriovenous difference in the lactate concentration by 100 and dividing it by the arterial lactate concentration.

**Statistical Analysis**

The time course of changes in hemodynamic parameters in each group was compared by 1-way repeated measures ANOVA, followed by the Fisher test. The time course of changes in hemodynamic parameters between groups was compared by repeated measures ANOVA, followed by the Fisher test. All values are expressed as mean±SEM, and P<0.05 was considered significant.

**Results**

**Effects of an Intracoronary Administration of Aldosterone on Systemic and Coronary Hemodynamics in the Nonischemic Hearts**

In the nonischemic hearts, either heart rate or CPP was not significantly changed during the infusion of aldosterone (Figure 1A and 1B). The infusion of vehicle did not change CBF throughout 60 minutes. Aldosterone at the dose of 0.1 nmol/L gradually decreased CBF from 5 minutes and reached the maximal decrease of CBF 30 minutes after the onset of hypoperfusion and did not further change CBF. Aldosterone at the dose of either 0.1 nmol/L or 0.2 nmol/L caused comparable decrease in CBF, but aldosterone at the dose of 0.05 nmol/L decreased CBF to a lesser extent than did 0.1 nmol/L (Figure 1C).

**Effects of an Intracoronary Administration of Aldosterone on Coronary Hemodynamics and Cardiac Functions in the Ischemic Hearts**

Before and during coronary hypoperfusion, both heart rate and CPP were unchanged with or without pharmacological interventions. There were no significant differences in baseline hemodynamics among all groups. The infusion of aldosterone (0.1 nmol/L) decreased CBF gradually from 5 minutes and reached maximal decrease at 30 minutes (Figure 2A). In the ischemic hearts, both FS (23.7±1.5% to 8.4±0.7%) and LER (41.4±3.0% to 31.7±2.9%) 30 minutes after the onset of hypoperfusion were decreased (P<0.05) compared with the baseline. Furthermore, the intracoronary infusion of aldosterone further decreased both FS (5.4±0.4%) and LER (41.4±3.7%) in the ischemic hearts. Co-administration of GF109203X completely blunted the aldosterone-induced decrease in CBF (38.1±2.9 mL/100g
per minute) (Figure 2A). This agent also blunted the aldosterone-induced decreased in both FS (8.3 ± 0.7%) and LER (−30.2 ± 1.3%) in the ischemic hearts (Figure 2B and 2C). The infusion of GF109203X alone (n = 5) did not change CBF (33.8 ± 3.7 to 34.5 ± 4.3 mL/100 g per minute), FS (8.4 ± 1.0 to 8.2 ± 1.0%), or LER (−32.4 ± 3.2 to −33.6 ± 2.8%) in the ischemic hearts. Co-administration of spironolactone (n = 5) did not prevent the aldosterone-induced decrease in CBF (24.0 ± 0.5 mL/100 g per minute). The infusion of bovine serum albumin-conjugated aldosterone decreased CBF gradually from 5 minutes and reached maximal decrease at 30 minutes (22.5 ± 0.9 mL/100 g per minute).

**Effects of Aldosterone on the Activation of PKC With and Without Ischemia**

As shown in Figure 3, in the nonischemic condition, aldosterone induced the translocation of PKCe from cytosolic to membrane fraction in the vascular segments of the LAD. Moreover, the ischemic insult itself induced the translocation of PKCe from cytosolic to membrane fraction and aldosterone further augmented the translocation of PKCe in the vascular segments under ischemia.

**Discussion**

We demonstrated here that the intracoronary administration of aldosterone rapidly decreased CBF in the ischemic as well as the nonischemic hearts in vivo. Moreover, aldosterone further worsened the contractile and metabolic functions gauged by FS and LER, respectively, in the ischemic hearts. These decreases in CBF, FS, and LER in the ischemic hearts were blunted by a PKC inhibitor but not an MR antagonist. In addition, bovine serum albumin-conjugated aldosterone reduced CBF under ischemic conditions, suggesting that the
reduction in CBF by aldosterone was mediated through possible membrane receptors, not intracellular MR. These results indicate that aldosterone nongenomically induces vasoconstriction via PKC-dependent pathways possibly through membrane receptors, which leads to the worsening of the cardiac contractile and metabolic function in the ischemic hearts.

**Rapid Aldosterone-Induced Coronary Vasoconstriction in the Nonischemic and Ischemic Hearts**

In this study, in the nonischemic hearts, the intracoronary administration of aldosterone decreased CBF within 30 minutes, suggesting that aldosterone nongenomically reduces CBF. Moreover, in the ischemic hearts, we observed the rapid coronary vasoconstriction induced by aldosterone along with the decrease in FS and LER, both of which indicated the contractile and metabolic deterioration, respectively. These findings suggest that the rapid decrease in CBF induced by aldosterone may cause the worsening of ischemia in the in vivo hypoperfused hearts.

**Involvement of PKC in the Aldosterone-Induced Coronary Vasoconstriction**

Aldosterone is reported to activate PKC in distal colon cells and cultured kidney cells, and to decrease its activity stimulated by phorbol-12-myristate-13-acetate in rat neonatal cardiomyocytes.12–14 In our study, this nongenomic effect of aldosterone on CBF was completely blunted by the PKC inhibitor, GF109203X, confirming the involvement of the PKC activation. The dose of aldosterone at 0.1 nmol/L was reported to increase intracellular Ca\(^{2+}\) in cultured rat and rabbit vascular smooth muscle cells.3 Consistent with this report, we showed that aldosterone activated Ca\(^{2+}\)-dependent PKCa in the vascular segments of the ischemic heart. There are some reports that endothelial nitric oxide synthase is a PKC substrate and PKC-mediated phosphorylation inhibits endothelium nitric oxide synthase activity.20,21 Because nitric oxide is widely known to be a vasodilative agent,22 decreased nitric oxide activity could attenuate the vascular tone, leading the decrease in CBF. Thus, there is a possibility that aldosterone induced vasoconstriction because of decreased endothelium nitric oxide synthase activity by PKC activation. Because we could not obtain antibodies that react with canine Ca\(^{2+}\) -independent subtypes of PKC, possible involvement of other subtypes of PKC was not investigated in the present study.

**The Possibility of Transmembrane Receptors of Aldosterone in Canine Hearts**

We demonstrated that spironolactone, a classical antagonist of intracellular MR, did not prevent aldosterone-induced vasoconstriction. In addition, bovine serum albumin–conjugated aldosterone induced vasoconstriction. Because bovine serum albumin–conjugated aldosterone would not permeate into the cytoplasm, the effects of bovine serum albumin–conjugated aldosterone on vascular tone were not mediated through intracellular MR, but rather possible membrane receptors. Arima et al suggested that aldosterone caused vasoconstriction in renal microcirculation mediated via membrane-bound receptors.23 Although further investigation to identify the transmembrane receptors directly will be needed, these findings might support the possibility of the presence of the transmembrane receptors. These coronary vasoconstriction effects of aldosterone were categorized into AII-b according to Mannheim classifications indicating direct steroid action via nonclassical receptors.24

**Perspectives**

Recent large clinical trials resolutely established the beneficial effects of chronic blockade of aldosterone receptor using for patients with chronic heart failure after myocardial infarction.25,26 In this study, we showed that in the ischemic hearts the nongenomic effect of aldosterone deteriorated ischemia and that this effect was blunted by the inhibition of PKC, not a MR antagonist. Our data suggest that elevated levels of aldosterone may worsen myocardial ischemia via nongenomic as well as genomic pathways in the ischemic hearts. Thus, we believe that this report throws a light on the novel clinical drug development to target nongenomic effects of aldosterone in the ischemic hearts, as well as the chronic inhibition of genomic effects of aldosterone using an antagonist against intracellular MR.

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