

Inhibitory Effect of Evodiamine on Aldosterone Release by Zona Glomerulosa Cells in Male Rats

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Abstract

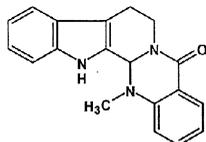
Evodiamine is a bioactive alkaloid extracted from a Chinese herb named Wu-Chu-Yu, which possesses thermoregulatory, analgesic, and cardiovascular effects. Some studies have demonstrated that evodiamine reduces blood pressure through acting on endothelium and smooth muscle cells to produce a vasodilatory effect, but whether it affects aldosterone secretion is unclear. The purpose of this study was to examine the effect of evodiamine on aldosterone release in adrenal zona glomerulosa (ZG) cells. ZG cells were isolated from the adrenal glands of adult male rats and incubated with angiotensin II (Ang II, 1×10^{-7} M) and ³H-pregnenolone in the presence or absence of evodiamine (1×10^{-6} – 1×10^{-3} M) at 37 °C for one hour. The concentration of aldosterone in the media was measured by a radioimmunoassay. The level of radioactivity incorporated into aldosterone and its precursors after incubation of ZG cells with ³H-pregnenolone was analyzed by thin-layer chromatography. The results demonstrated that evodiamine decreased the basal level of and Ang II-induced release level of aldosterone in rat ZG cells. Administration of evodiamine also decreased the level of radioactivity incorporated into ³H-corticosterone and ³H-aldosterone following incubation of ZG cells with ³H-pregnenolone. This suggests that evodiamine affects aldosterone release in rat adrenal glomerulosa cells by acting on Ang II-associated pathway and reducing the activity of 11 β -hydroxylase (an enzyme which converts deoxycorticosterone to corticosterone) during the steroidogenesis of aldosterone.

Key Words: evodiamine, zona glomerulosa cells, aldosterone, 11 β -hydroxylase

Introduction

Wu-Chu-Yu, the dry fruits of *Evodia rutaecarpa* (Juss.) Benth., is a traditional Chinese herb officially listed in the Chinese Pharmacopoeia and has been used as an analgesic, antiemetic, and astringent, and in the treatment of hypertension for hundreds of years (15). It contains several alkaloids, such as rutaevine, evodiamine, rutaecarpine, wuchuyine and rhetsinine (2). Evodiamine (Fig. 1) is an active alkaloid isolated by Asahina *et al.* more than 70 years ago and widely studied recently. It has been reported that evodiamine

has body temperature retention effect in hypothermic rats (9,16). In isolated rat aorta, evodiamine has a vasodilatory effect and the effect is endothelium dependent (6). Another study showed that evodiamine inhibited calcium influx in vascular smooth muscle cells via blocking the receptor-mediated calcium channel to generate a vasorelaxant effect, therefore lowering hypertension (5). Evodiamine also has a diuretic effect which can control blood volume (3). Evodiamine also affects testosterone secretion by inhibiting the cAMP-associated pathway and 17 β -hydroxysteroid dehydrogenase (17 β -HSD) (12).



Evodiamine (C₁₉H₁₇N₃O, MW : 303.35)

Fig. 1. The chemical structure of evodiamine isolated from Wu-Chu-Yu.

These studies demonstrated that evodiamine is one of the main active components of Wu-Chu-Yu and affects body temperature, blood pressure, and the endocrine system.

Blood pressure is regulated by both the cardiovascular and the endocrine system. The regulator of blood pressure in the endocrine system is aldosterone. Aldosterone is a steroid hormone secreted by the adrenal glomerulosa cells and maintains normal blood volume and the sodium balance through acting on the distal tubules and the cortical collecting ducts of the kidneys (17). The important factors controlling aldosterone secretion are the renin-angiotensin system, adrenocorticotropin and potassium (1). Calcium, sodium, serotonin, endothelin, vasopressin, catecholamine and prostaglandin also affect the secretion of aldosterone (8).

Previous studies have shown that evodiamine acts on both the vessel endothelial cells and smooth muscle cells to reduce blood pressure (5,6). The effect of evodiamine on the production of adrenal mineralocorticoid is not known. This study is to explore whether evodiamine can affect aldosterone secretion by acting directly on rat ZG cells.

Materials and Methods

Animals

Male Sprague-Dawley rats (300~350 g) were provided by the animal center of National Yang-Ming University and were housed in a temperature-controlled room (22 ± 1°C) with 14 h of artificial illumination daily (0600~2000) and given water and food ad libitum.

Effects of Evodiamine on Aldosterone Release by Rat ZG Cells

Rat ZG cells were prepared by the method previously described (10,11). After preincubation with Krebs-Ringer bicarbonate buffer containing K⁺ 3.6 mM, glucose 11.1 mM, and bovine serum albumin (BSA) 0.2 % (KRBGA medium) at 37 °C in a shaker bath (50 cycles per min) aerated with 95 % O₂ and 5 % CO₂, aliquots (1 ml) of the ZG cells (5 × 10⁴ cells)

were incubated with evodiamine (1 × 10⁻⁶~1 × 10⁻³ M) for 1 h. The evodiamine was provided by the National Research Institute of Chinese Medicine and was dissolved in dimethyl sulfoxide (Wako Pure Chemical Industries, Ltd., Osaka, Japan). To examine the effect of evodiamine on angiotensin II (Ang II)-stimulated aldosterone release, ZG cells were incubated with Ang II (1 × 10⁻⁷ M, Sigma, St. Louis, MO, USA) in the presence or absence of evodiamine for 1 h. To investigate the effect of evodiamine on the steroidogenesis of aldosterone, ZG cells were incubated with ³H-pregnenolone (Amersham International plc., Buckinghamshire, UK) in the presence or absence of evodiamine. At the end of incubation, 0.2 ml of ice-cold KRBGA medium was added to stop the reaction. The media were collected and stored at -20 °C for either aldosterone RIA or thin layer chromatography (TLC).

RIA of Aldosterone

Antiserum No. PHH-066 to aldosterone was generated by the method previously described (12). The antiserum was produced by immunizing the rabbit with 4-pregnen-11β, 21-diol-3,18,20-trione 3CMO: BSA conjugate (Steraloids Inc., USA). With this antiserum, a RIA was established for the measurement of aldosterone levels in the plasma and medium samples. In this RIA system, a known amount of unlabeled aldosterone or an aliquot of plasma extracts or medium samples adjusted to 0.3 ml by buffer solution (1 % BSA-borate buffer, pH 7.8) then incubated with 0.1 ml of aldosterone antiserum (1:6000) diluted with buffer solution and 0.1 ml of ³H-aldosterone (approximately 8,000 cpm, Amersham, UK) at 4 °C for 24 h. Duplicate standard curves with 5 points ranging from 4 to 1200 pg of aldosterone were incubated in each assay. The assay tubes were added with 0.1 ml of 0.5 % dextrane-coated charcoal (Sigma, USA) and the mixture incubated on ice for 15 min. The supernatant was mixed with 3 ml liquid scintillation fluid (Ready Safe, Beckman, Fullerton, CA, USA) before counting the radioactivity in an automatic beta counter (Wallac 1409, Pharmacia, Turku, Finland). The maximum binding of ³H-aldosterone with anti-aldosterone antiserum No. PHH-066 was 37 %. The sensitivity of aldosterone RIA was 5 pg per assay tube. The inhibition curves produced by the ether-extracted rat plasma and the incubation media of rat ZG cells were parallel to that given by unlabeled aldosterone (Fig. 2.). The cross-reactivity of various steroids with the anti-aldosterone antiserum are shown in Table 1. The intra- and interassay coefficients of variance were 4.0 % (n = 5) and 8.7% (n = 5).

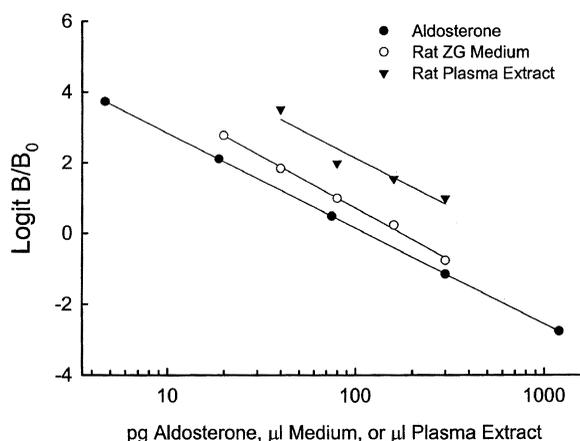


Fig. 2. Dose-dependent curves for rat aldosterone, rat plasma, and rat zona glomerulosa (ZG) cell medium after a log-ligand transformation in an aldosterone radioimmunoassay system by using anti-aldosterone serum No. PHH-066.

Thin Layer Chromatography (TLC)

The technique of thin layer chromatography was a modification of the method of Blomquist *et al.* (11). ZG cells were incubated with ^3H -pregnenolone (10,000 cpm, 5 pmol, Amersham International plc, Buckinghamshire, UK) in the presence or absence of evodiamine for 1 h. The medium was collected, extracted by diethyl ether, and then quickly frozen in a mixture of acetone and dry ice. The diethyl ether layer was collected, dried, and reconstituted in 100 μl 100% ethanol containing 5 μg of each of the carriers, including progesterone, deoxycorticosterone (DOC), corticosterone, and aldosterone. Aliquots (50 μl) of samples were applied to a TLC plate (0.25 μg thick silica G sheets precoated with fluorescent indicator, Macherey-Nagel, Duren, Germany) then developed in a mixture of carbon tetrachloride and acetone (4:1; vol/vol). The sheets were dried and the locations of steroid-containing spots were visible under UV light. The R_f values were 0.97 for progesterone, 0.68 for DOC, 0.24 for corticosterone, and 0.11 for aldosterone. The spots were excised and transferred into vials containing 1 ml of liquid scintillation fluid (Ready Safe, Beckman, USA) before the radioactivity was counted in an automatic beta counter (Wallac 1409, Pharmacia, Turku Finland).

Statistical Analysis

All data was presented as mean \pm SEM. The homogeneity of the means was tested by analysis of variance, and the significance of difference between specific means was tested by Duncan's multiple range test (13). The difference between two means was considered to be statistically significant when P was

Table 1. Relative Cross-Reactivities of Variant Steroids with Anti-Aldosterone Serum No. PHH-066 at 1:4000 Dilution.

Steroid Name	Cross Reactivity (%)
Aldosterone	100
Androstenedione	0.03
Deoxycorticosterone	0.03
17 α -Hydroxyprogesterone	0.03
Corticosterone	0.02
Cortisone	0.01
11-Deoxycortisol	0.01
Estrone	0.01
17 α -Estradiol	0.01
Estriol	0.01
Progesterone	0.01
Testosterone	0.01
Androstenediol	<0.01
Cholesterol	<0.01
Cortisol	<0.01
5 α -Dihydrotestosterone	<0.01
17 β -Estradiol	<0.01
Pregnenolone	<0.01

less than 0.05.

Results

Effect of Evodiamine on Basal and Ang II-Stimulated Aldosterone Release

Figure 3 illustrates the results of basal and Ang II (1×10^{-7} M)-stimulated aldosterone release by ZG cells. Evodiamine dramatically decreased aldosterone release by ZG cells in a dose-dependent manner. Evodiamine at 1×10^{-4} M reduced aldosterone release by 80 % compared to control group ($P < 0.01$). Administration of 1×10^{-3} M evodiamine almost totally inhibited aldosterone secretion in ZG cells.

Ang II remarkably elevated aldosterone release (228%) in ZG cells compared to the control group ($P < 0.05$). Evodiamine at 1×10^{-6} – 1×10^{-3} M significantly decreased Ang II-stimulated aldosterone release ($P < 0.05$ or $P < 0.01$) in a dose-dependent manner (Fig. 3). The dose-dependent effect of evodiamine on Ang II-stimulated aldosterone release is stronger than on the basal release of aldosterone (Fig. 3).

Effect of Evodiamine on The Steroidogenesis of Aldosterone in ZG Cells

Four H^3 -labeled steroid products including ^3H -progesterone, ^3H -deoxycorticosterone (DOC), ^3H -corticosterone, and ^3H -aldosterone have been detected

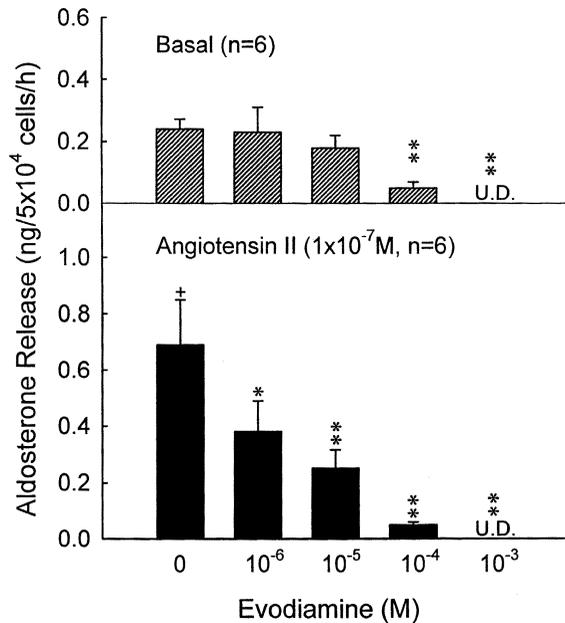


Fig. 3. Effects of evodiamine on basal- and angiotensin II (10^{-7} M)-stimulated aldosterone release by zona glomerulosa (ZG) cells in male rats. *, **, $P < 0.05$ and $P < 0.01$ as compared with evodiamine = 0 M, respectively. †, $P < 0.05$ as compared with the basal group. Each value represents the mean \pm SEM. U.D.= undetectable.

after incubation of rat ZG cells with ^3H -pregnenolone. The radioactivity of these products has been used to measure the enzyme activity during steroidogenesis of aldosterone in ZG cells. As shown in Figure 4, incubation of evodiamine at 1×10^{-4} M and 1×10^{-3} M with rat ZG cells increased the accumulation of ^3H -progesterone ($P < 0.01$). Evodiamine at 1×10^{-3} M also increased product of ^3H -deoxycorticosterone ($P < 0.01$). However, the administration of 1×10^{-3} M evodiamine reduced accumulation of ^3H -corticosterone by $62.9 \pm 4.1\%$ and of ^3H -aldosterone by $59.8 \pm 4.3\%$. There was no significant difference between the decrease in ^3H -corticosterone and the decrease in ^3H -aldosterone caused by evodiamine. These results demonstrated that evodiamine inhibited the enzyme activity of the 11 β -hydroxylase, but not the aldosterone synthases, in rat ZG cells.

Discussion

It has been shown that administration of evodiamine on testicular interstitial cells decreases the secretion of testosterone (12). However, the effects of evodiamine on other endocrine systems is not known. The present study focuses on the effect of evodiamine on the steroidogenesis of aldosterone in rat ZG cells. Fig. 3 showed that evodiamine reduces dramatically aldosterone release from ZG cells, indicating that the biosynthesis of aldosterone may be

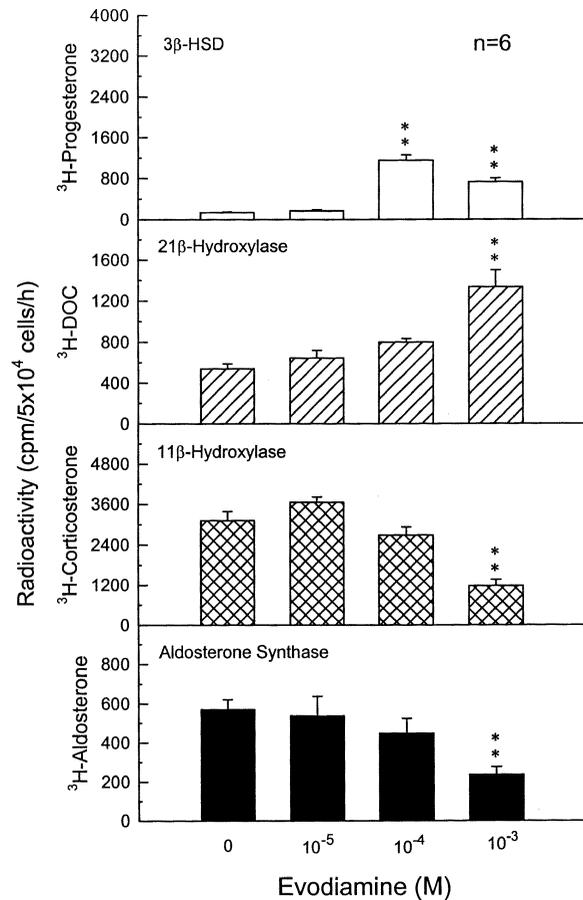


Fig. 4. Effects of evodiamine (10^{-5} – 10^{-3} M) on the level of radioactivity of steroidogenic products following incubation of rat ZG cells with ^3H -pregnenolone at 37°C for 1 h. **, $P < 0.01$ as compared with evodiamine = 0 M. Each value represents the mean \pm SEM.

affected.

It has been well known that the biosynthesis of aldosterone is mainly regulated by the renin-angiotensin system (7,17). Ang II stimulates aldosterone secretion by increasing calcium influx and mobilizing intracellular calcium (4). In the present study rat ZG cells were incubated with or without Ang II to examine if evodiamine affects Ang II-induced aldosterone release. The results suggest that evodiamine affects aldosterone secretion by acting directly on the Ang II-associated pathway. This suggests that evodiamine might inhibit calcium influx by blocking the calcium channel and/or affecting intracellular calcium mobilization. On the other hand, evodiamine has been reported to lower blood pressure through a vasodilatory effect on isolated rat aorta (6). Evodiamine also inhibits calcium influx in vascular smooth muscle cells via blocking the receptor-mediated calcium channel to generate a vasorelaxant effect and therefore lowering hypertension (5). Our studies suggest that evodiamine may have contribute

to lowering blood pressure via a decrease in aldosterone secretion through an inhibition of Ang II-associated pathway.

During steroidogenesis, cholesterol is firstly converted to pregnenolone by P450 side-chain cleavage enzyme (P450_{scc}), and then converted to progesterone by 3 β -hydroxysteroid dehydrogenase (3 β -HSD), deoxycorticosterone by 21 β -hydroxylase, corticosterone by 11 β -hydroxylase, and finally converted to aldosterone by aldosterone synthase (7, 8, 14). To examine whether evodiamine affect the enzymes of steroidogenesis of aldosterone, ³H-pregnenolone was used to observe the biosynthesis of aldosterone. Our data demonstrated that the production of aldosterone was affected by evodiamine. The increased accumulations of ³H-progesterone and ³H-deoxycorticosterone by evodiamine imply that the process of conversion of ³H-deoxycorticosterone to ³H-corticosterone is blocked stopping conversion to the downstream products. These results indicate that at least 11 β -hydroxylase is inhibited by 10⁻³ M evodiamine. Since the evodiamine-induced reduction of ³H-aldosterone was similar to that of ³H-corticosterone, we speculate that the activities of the aldosterone synthases (i.e. 18-hydroxylase and 18-OH-dehydrogenase) are not altered by evodiamine.

In conclusion, these results suggest that evodiamine decreases aldosterone release in rat ZG cells, at least in part, affecting the Ang II-associated pathway and 11 β -hydroxylase activity in steroidogenesis.

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