Effects of Evodiamine on the Secretion of Testosterone in Rat Testicular Interstitial Cells

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Evodiamine, a bioactive component isolated from the Chinese medicine Wu-chu-yu, exhibits vasodilative and antianoxic action. Although evodiamine indeed has many biological effects, its effects on the endocrine system are not clear. The present study explored the effects of evodiamine on testosterone secretion in vitro. Rat collagenase-dispersed testicular interstitial cells (TICs) were incubated with evodiamine (0 to 10^{-4} mol/L) in the presence or absence of human chorionic gonadotropin (hCG), forskolin, 8-bromo-adenosine 3′:5′-cyclic monophosphate (8-Br-cAMP), or steroidogenic precursors (including 25-hydroxycholesterol, pregnenolone, progesterone, 17α-hydroxyprogesterone, and androstenedione) at 34°C for 1 hour. The testosterone concentration in the media samples was measured by radioimmunoassay. Evodiamine 10^{-4} mol/L was effective to reduce both basal and hCG-stimulated testosterone secretion in rat TICs after 1, 2, or 4 hours of incubation. The stimulatory effect of forskolin on testosterone release in TICs was prevented by administration of evodiamine. Evodiamine 10^{-4} mol/L also decreased 8-Br-cAMP- and androstenedione-stimulated testosterone secretion. These results suggest that evodiamine reduces testosterone secretion in rat TICs via a mechanism involving reduced activity of cAMP-related pathways and 17β-hydroxysteroid dehydrogenase (17β-HSD).

EVODIAMINE, isolated from the dry unripened fruit of *Evodia rutaecarpa Bentham* (a Chinese medicine named Wu-chu-yu), has been reported to stimulate the secretion of catecholamines from perfused bovine adrenal medulla[1] (Fig 1). It was also found to affect vasotension, anoxia, and the body temperature.[2-4] The thermoregulatory effects of evodiamine were investigated by the intraperitoneal injection in rats of 2.5 to 10 mg evodiamine/kg body weight.[4] High-performance liquid chromatography has also been used in a pharmacokinetic study of evodiamine in rats after intravenous administration of 2 mg/kg body weight.[5] Recently, we found that some traditional Chinese medicines, such as bufalin, Chansu, and digoxin, have inhibitory effects on peripheral testosterone production.[6-8] The inhibition by these Chinese medicines might occur through different specific sites in rat testicular interstitial cells (TICs). Although evodiamine has many biological functions, the relationship between its action and endocrine function is unclear. The intracellular mechanism by which evodiamine mediates steroidogenesis has not been established either. A number of in vitro studies have shown that many compounds may directly or indirectly target the enzymes required for the biosynthesis of testosterone in Leydig cells, eg, 17β-hydroxysteroid dehydrogenase (17β-HSD).[9,10] The biosynthesis of steroid hormones by Leydig cells requires the sequential actions that convert cholesterol into various steroid classes.[9] The final step is the reduction of androstenedione to testosterone via the activity of 17β-HSD. This step is reversible with substrate and product concentrations regulating the direction of the reaction.[11]

The present study was performed to examine the effect of evodiamine on basal and human chorionic gonadotropin (hCG)-stimulated testosterone secretion in rat TICs. We also examined whether the steroidogenic enzymes in TICs are involved in evodiamine's effects on the production of testosterone. We found that evodiamine inhibited testosterone production through mechanisms involving a decrease of 17β-HSD activity in TICs.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 300 to 350 g were housed in a temperature-controlled room (22° ± 1°C) with 14 hours of artificial illumination daily (6 AM to 8 PM) and food and water available ad libitum.

Preparation of TICs

Collagenase-dispersed TICs were prepared with a procedure described elsewhere.[8] The cell concentration (1×10^6/mL), cell viability (>97%), and sperm cell count (<5%) were determined using a hemocytometer and the trypan blue method. Total cell proteins were determined by the method of Lowry et al.[12] The abundance of Leydig cells in our preparation was measured by the 3β-HSD staining method,[13,14] and this preparation was found to contain approximately 18% ± 2% Leydig cells.

Effects of Evodiamine on Testosterone Secretion in Rat TICs

Aliquots (1 mL) of cell suspensions (1×10^6/mL) were preincubated with incubation medium in polyethylene tubes for 1 hour at 34°C under a controlled atmosphere (95% O_2 and 5% CO_2), shaken at 100 rpm. The supernatant fluid was decanted after centrifugation of the tubes at 100× *g* for 10 minutes. Evodiamine (10^{-6} to 10^{-4} mol/L), hCG (0.05 IU/mL), or hCG + evodiamine in 200 μL fresh medium were then added to the tubes. After 1, 2, or 4 hours of incubation, 2 mL ice-cold PBSG buffer (0.1% gelatin in 0.01 mol/L phosphate buffer and 0.15 mol/L sodium chloride, pH 7.5) was added to stop the incubation. The spent medium was centrifuged at 100× *g* and stored at −20°C until analysis for testosterone by radioimmunoassay.

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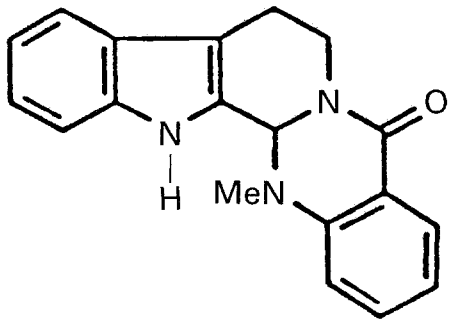

Evodiamine: $C_{19}H_{17}N_3O$ M.W. 303.35

Fig 1. Structure and molecular weight of evodiamine.

To further study the effects of cAMP and steroidogenic precursors on the production of testosterone, aliquots (1 mL) of cell suspensions were preincubated and primed for 30 minutes with or without forskolin, an adenylyl cyclase activator (10^{-6} mol/L; Sigma, St Louis, MO), and then incubated for 1 hour with forskolin (10^{-6} mol/L), 8-Br-cAMP, a cAMP analog (10^{-4} mol/L; Sigma), or steroidogenic precursors (10^{-5} or 10^{-7} mol/L, including 25-hydroxycholesterol, pregnenolone, progesterone, 17α-hydroxyprogesterone, and androstenedione; Sigma) in the presence or absence of evodiamine. At the end of the incubation, 2 mL ice-cold PBSG buffer was added, immediately followed by centrifugation at 100× *g* for 10 minutes at 4°C. The supernatant was stored at −20°C until analysis for testosterone by radioimmunoassay.

Radioimmunoassay of Testosterone

The testosterone concentration was determined by radioimmunoassay as described previously.[15,16] With antitestosterone serum no. W8 (produced and characterized by our laboratory), the sensitivity of the testosterone radioimmunoassay was 2 pg per assay tube. The intraassay and interassay coefficient of variation was 10.6% (n = 5) and 6.2% (n = 7), respectively.

Materials

Bovine serum albumin, HEPES, Hanks balanced salt solution, medium 199, sodium bicarbonate, penicillin G, streptomycin, heparin, collagenase, hCG, forskolin, 8-Br-cAMP, 25-hydroxycholesterol, pregnenolone, progesterone, 17α-hydroxyprogesterone, and androstenedione were purchased from Sigma. Evodiamine (Fig 1) was prepared and provided by the National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China. The drug dosages are expressed as the final molar concentration in the flask.

Statistical Analysis

All values are presented as the mean ± SEM. In some cases, the treatment means were tested for homogeneity by a two-way ANOVA, and the difference between specific means was tested for significance by Duncan's multiple-range test.[17] In other cases, Student's *t* test was used. A difference between two means was considered statistically significant at a *P* level less than .05.

RESULTS

Effects of Evodiamine on Testosterone Release In Vitro

Administration of evodiamine (10^{-4} mol/L) resulted in a significant decrease ($P < .05$ or $P < .01$) in testosterone release by TICs after 1, 2, or 4 hours of incubation. hCG (0.05 IU/mL) increased testosterone production by fivefold in the TICs. One hour of incubation of rat TICs was effective to explore both basal and hCG-stimulated testosterone release. In the presence of hCG, evodiamine (10^{-4} mol/L) inhibited testosterone release ($P < .05$ or $P < .01$) (Fig 2).

Effects of Evodiamine on cAMP-Regulated Testosterone Secretion

Both forskolin (10^{-6} mol/L) and 8-Br-cAMP (10^{-4} mol/L) caused a significant increase of testosterone secretion by TICs. The stimulatory effect of forskolin on testosterone release was prevented by administration of evodiamine (10^{-4} mol/L). Testosterone release in response to forskolin and 8-Br-cAMP was lower ($P < .01$) in the evodiamine-treated group versus the vehicle group (Figs 3 and 4).

Effects of Evodiamine on Steroidogenic Enzyme Activity

Steroidogenic precursors, including 25-hydroxycholesterol, pregnenolone, progesterone, 17α-hydroxyprogesterone, and androstenedione (10^{-5} or 10^{-7} mol/L), increased the release of testosterone by TICs (Fig 5). Evodiamine 10^{-4} mol/L significantly ($P < .01$) decreased the production of testosterone facilitated by both doses of androstenedione. Apparently, the activity of 17β-HSD was decreased by evodiamine.

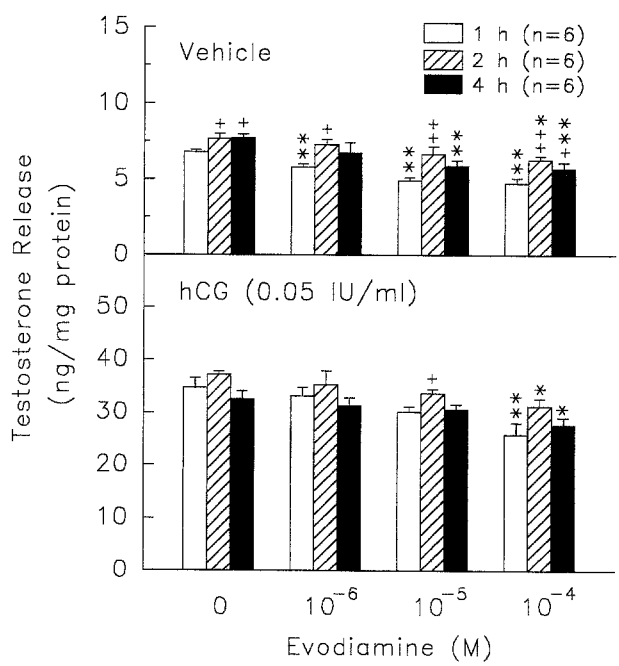

Fig 2. Release of testosterone by TICs in the presence or absence of hCG (0.05 IU/mL) after 1, 2, or 4 hours of incubation with different doses of evodiamine (mol/L). *$P < .05$, **$P < .01$ *v* evodiamine 0 mol/L. +$P < .05$, ++$P < .01$ *v* 1 hour. Results are the mean ± SEM.

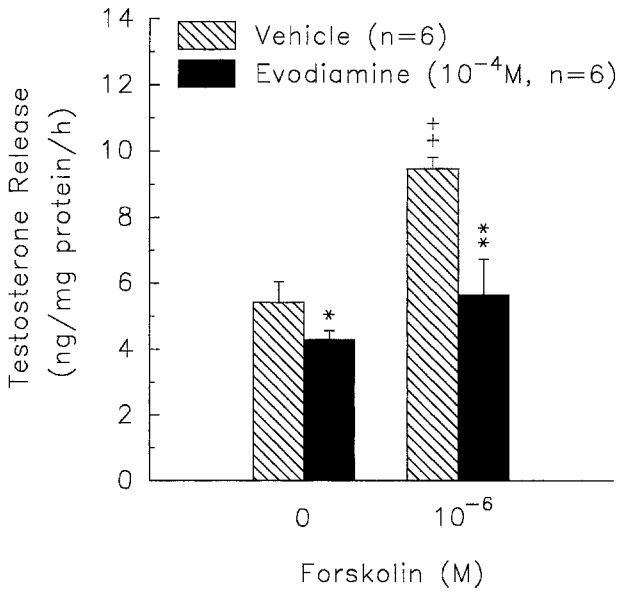

Fig 3. Effects of evodiamine (10^{-4} mol/L) on testosterone release by TICs after priming and incubation with or without forskolin (10^{-6} mol/L). *$P < .05$, **$P < .01$ *v* vehicle. ++$P < .01$ *v* forskolin 0 mol/L. Results are the mean ± SEM.

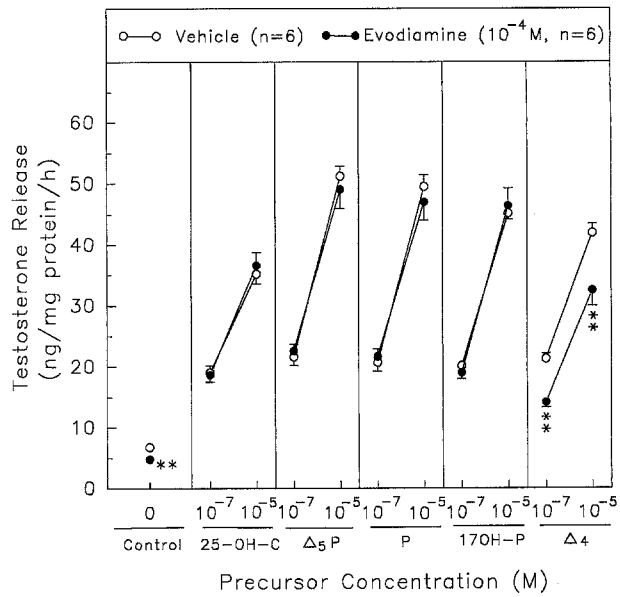

Fig 5. Effects of evodiamine (10^{-4} mol/L) on testosterone release by TICs after incubation with or without 25-hydroxycholesterol, pregnenolone, progesterone, 17α-hydroxyprogesterone, and androstenedione (10^{-5} or 10^{-7} mol/L). **$P < .01$ *v* vehicle. Results are the mean ± SEM.

DISCUSSION

Wu-chu-yu is a Chinese traditional medicine used for a syndrome characterized by cold hands and feet as a systemic symptom and migraines and vomiting as characteristic symptoms. Evodiamine is one of the major components of Wu-chu-yu. Previous studies have shown that evodiamine affects vasotension, anoxia, and body temperature.[2-4] Meanwhile, the possible antiinflammatory effects of evodiamine were examined by assessing the effects on nitric oxide production in the murine macrophage.[18] This evidence indicates that evodiamine indeed influences many organs and tissues, and may become a useful medicine in the future. The present study demonstrated that administration of evodiamine diminishes testosterone secretion by rat TICs.

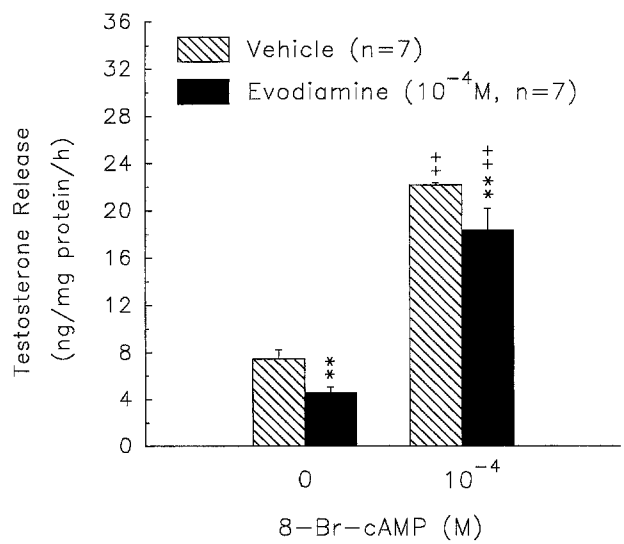

Fig 4. Effects of evodiamine (10^{-4} mol/L) on testosterone release by TICs after incubation with or without 8-Br-cAMP (10^{-4} mol/L). **$P < .01$ *v* vehicle. ++$P < .01$ *v* 8-Br-cAMP 0 mol/L. Results are the mean ± SEM.

It has been well established that hCG stimulates testosterone secretion both in vivo[15,16,19,20] and in vitro[15,16,21-23] via an increase in cAMP production.[15,16,24-26] In the present study, we found that not only basal but also hCG-stimulated testosterone production in vitro were diminished by evodiamine treatment (Fig 2). Forskolin, an adenylyl cyclase activator, and 8-Br-cAMP, a cAMP analog, effectively stimulated the production of testosterone in TICs, but the stimulation was inhibited by evodiamine (Figs 3 and 4). Administration of both forskolin and 8-Br-cAMP did not reverse the inhibition by evodiamine, which indicates that the inhibition may be located at not only the post–adenylyl cyclase but also the post-cAMP pathway of testosterone biosynthesis in rat TICs.

The biosynthesis of steroid hormones by Leydig cells requires the sequential actions that convert cholesterol into various steroid classes.[9] The interconversion of androstenedione to testosterone is catalyzed by the microsomal enzyme 17β-HSD. This step is reversible, while others are irreversible and significantly alter the activities and functions of the C_{19} substrate.[11] Evodiamine inhibited the conversion of testosterone from androstenedione (Fig 5), the substrate of 17β-HSD, indicating that evodiamine reduced the activity of 17β-HSD. Meanwhile, testosterone production in response to other precursors (25-hydroxycholesterol, pregnenolone, progesterone, 17α-hydroxyprogesterone, and androstenedione) was not altered by evodiamine, and one of the reasons may be the balance between different substrates and products. These phenomena finally

caused the modest decrease in testosterone secretion. These results suggest that evodiamine inhibited testosterone secretion in rat TICs, at least in part, by reduction of 17β-HSD activity in testosterone steroidogenesis.

Previously, we found that administration of either bufalin or Chansu extracts in male rats diminished the luteinizing hormone (LH) response to gonadotropin-releasing hormone (GnRH).[6,7] Whether the secretion of GnRH by the hypothalamus and LH by the anterior pituitary are altered by evodiamine is not yet known, but would be interesting to explore in the future.

In summary, the present results demonstrate that evodiamine decreased testosterone production partly by inhibition of the activity of cAMP-related pathways and 17β-HSD in rat TICs.

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