Inhibition of Testosterone Secretion by *S*-Petasin in Rat Testicular Interstitial Cells

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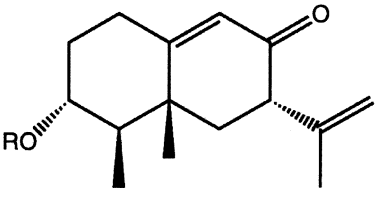
Abstract

***S*-petasin, a kind of sesquiterpene ester, is the anti-inflammatory and analgesic component of the butterbur (*Petasites hybridus*). The clinical benefit of *S*-petasin is the spasmolytic activity, but its side effects on the reproductive endocrinology are not clear yet. The present study was to explore the effects of *S*-petasin on the secretion of testosterone in vivo and in vitro. We found that single intravenous injection of *S*-petasin (1 μg/kg) decreased basal plasma testosterone concentration in adult male rats. The enzymatically dispersed rat testicular interstitial cells were incubated with *S*-petasin (0~4.3×10^{-5}M) in the presence or absence of human chorionic gonadotropin (hCG, 0.05 IU/ml), forskolin (adenylyl cyclase activator, 10^{-5} M), and androstenedione (testosterone biosynthesis precursor, 10^{-9} M) at 34 °C for 1 h. The concentrations of testosterone in the incubation medium were measured by radioimmunoassay. *S*-petasin at 4.3×10^{-7} M was effective to reduce the basal and hCG-stimulated release of testosterone in rat testicular interstitial cells. The stimulatory effects of testosterone secretion induced by forskolin and androstenedione were significantly reduced by *S*-petasin at 4.3×10^{-5} M and 4.3×10^{-6} M, respectively. These results suggest that *S*-petasin inhibits the production of testosterone in rat testicular interstitial cells in part through diminishing the activities of adenylyl cyclase and 17-ketosteroid reductase.**

Key Words: testosterone, *S*-petasin, rat, testicular interstitial cells

Introduction

It has been known that *S*-petasin (Fig. 1) possesses many biological effects including tonsillitis, spasmolytic agent of the gastrointestinal tract and asthmatic attacks (4). However, the relationship between the action of S-petasin and reproductive endocrinology is not known. The intracellular mechanism by which *S*-petasin mediates steroidogenesis has not yet been established. 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, including cholesterol side chain cleavage enzymes (cytochromes P450scc), 17α-hydroxylase/C17-C20 lyase (P450c17), 3β-hydroxysteroid dehydrogenase (3β-HSD), and 17-ketosteroid reductase (17-KSR) (11). The biosynthesis of steroid hormones by Leydig cells requires the sequential actions that convert cholesterol into various steroid classes (11). The catalysis of androstenedione by microsomal enzyme 17-KSR is the last step to produce testosterone.



S-Petasin ($C_{19}H_{26}O_3S$, MW=334)
R=COCH=CH(SMe) *cis*-

Fig. 1. Structure and molecular weight of S-petasin.

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The present study was carried out to examine the effects of *S*-petasin on the basal and human chorionic gonadotropin (hCG)-stimulated secretion of testosterone in rat testicular interstitial cells. The probable action sites of *S*-petasin were also investigated. We found that *S*-petasin inhibited testosterone production in part through diminishing the activities of adenylyl cyclase and 17-ketosteroid reductase.

Materials and Methods

Animals

Male rats of the Sprague-Dawley strain weighing 300-350 g were housed in a temperature controlled room (22±1 °C) with 14 h of artificial illumination daily (06 h 00 min-20 h 00 min) and given food and water *ad libitum*.

In Vivo Experiment

Male rats were anaesthetized with ether and then catheterized *via* the right jugular vein (19, 20). Twenty hours later, the conscious rats were injected intravenously with vehicle (saline, 1 ml/kg), *S*-petasin (1 μg/ml/kg), *via* the jugular catheter. Blood samples (0.5 ml each) were collected at 0 and 30 min after the challenge. Plasma was separated by centrifugation at 10,000× g for 1 min. The concentration of testosterone in each plasma sample was measured by radioimmunoassay (RIA) after ether extraction.

Preparation of Testicular Interstitial Cells

The preparation of collagenase-dispersed of testicular interstitial cells was modified from the procedure described elsewhere (7). Cell concentration (1×10^6 cells/ml), viability (over 97%), and the sperm cells (less than 5%) were determined using a haemocytometer and the trypan blue method (7). After incubation, the cells were digested by 1 N NaOH and determined the protein content by the method of Lowry *et al*. (8). The abundance of Leydig cells in our preparation was measured by the 3β-HSD staining method (5,6) and this preparation was found to contain approximately 18±2% Leydig cells.

Effects of S-petasin on Testosterone Production

Aliquots (1 ml) of cell suspensions (1×10^6 cells/ml) were preincubated with incubation medium in polyethylene tubes for one hour at 34°C under a controlled atmosphere (95% O_2 and 5% CO_2), shaken at 100 cycles/min. The supernatant fluid was decanted after centrifugation of the tubes at 100× g for 10 min. *S*-petasin (0 ~ 4.3×10^{-5} M, i.e. 14.4 μg/ml), hCG (0.05 IU/ml), or hCG plus *S*-petasin in 200 μl fresh medium was then added to the tubes. For studying the activity of adenylyl cyclase in response to *S*-petasin, aliquots (1 ml) of cell suspensions (1.0×10^6 cells/ml) were primed for 30 min with forskolin (adenylyl cyclase activator, 10^{-5} M, Sigma, USA). *S*-petasin (0 ~ 4.3×10^{-5} M) with or without forskolin in 200 μl fresh medium were then added to the tubes. After 1 h of incubation, 2 ml ice-cold PBSG buffer (0.1% gelatin in 0.01 M phosphate buffer, 0.15 M 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 analyzed for testosterone by RIA.

Effects of S-petasin on Biosynthesis Pathway of Testosterone

Cell suspensions were preincubated for 1 h and then were incubated for 1 h with S-petasin (0 ~ 4.3×10^{-5} M) in the presence or absence of androstenedione (10^{-9} M, steroidogenic precursor). At the end of the incubation, 2 ml ice-cold PBSG buffer were added and immediately followed by centrifugation at 100× g for 10 min at 4 °C. The supernatant fluid was stored at -20 °C until analyzed for testosterone by RIA.

RIA of Testosterone

The concentrations of testosterone were determined by RIA as described previously (19,20). With anti-testosterone serum No. W8, the sensitivity of testosterone RIA was 2 pg per assay tube. The intra- and interassay coefficients of variation (CV) were 4.1% (n=6) and 4.7% (n=10), respectively.

Materials

Bovine serum albumin (BSA), N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid (HEPES), Hank's balanced sodium salt (HBSS), medium 199, sodium bicarbonate, penicillin-G, streptomycin, heparine, collagenase, human chorionic gonadotropin (hCG), forskolin, and androstenedione were purchased from Sigma Chemical Co. (St. Louis, MO, USA). S-petasin was obtained from National Research Institute of Chinese Medicine, Taiwan, ROC. [^{3}H]-testosterone was obtained from Amersham International Plc. (Bucks, UK). The doses of drugs were expressed in their final molar concentrations in the flask.

Statistical Analysis

All values are given as the mean±standard error of the mean (s. e. mean). In some cases, the means of

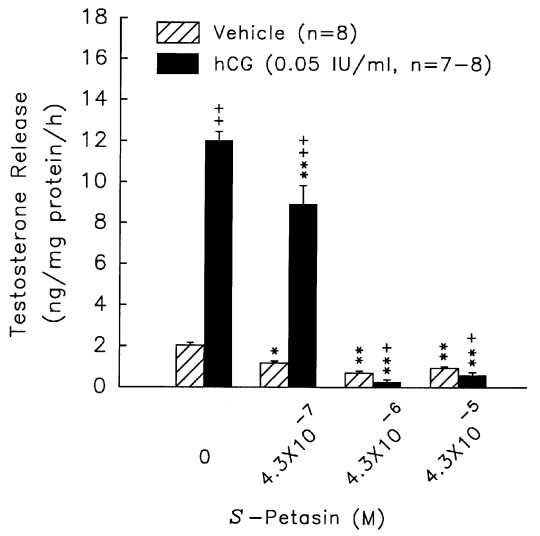

Fig. 2. Effects of *S*-petasin (0 ~ 4.3×10^{-5} M) on testosterone release *in vitro* in rat testicular interstitial cells pretreated with vehicle (hatched columns) or hCG (0.05 IU/ml, solid columns). Each column represents mean±s.e. mean. + $P<0.05$ and ++ $P<0.01$ compared with vehicle group. *$P<0.05$ and **$P<0.01$ compared with *S*-petasin at 0 M.

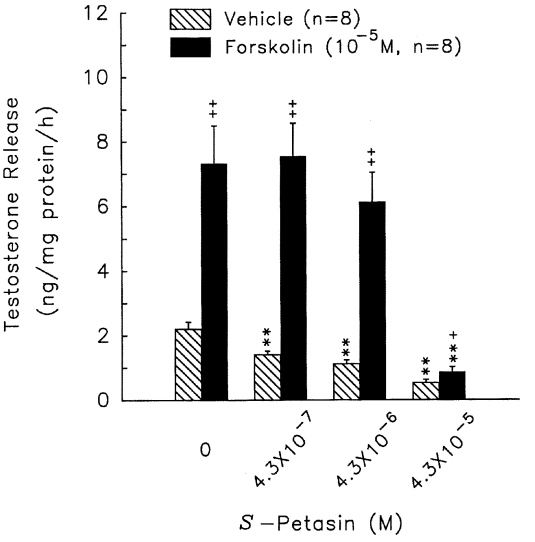

Fig. 3. Effects of *S*-petasin (0 ~ 4.3×10^{-5} M) on the testosterone release *in vitro* in rat testicular interstitial cells pretreated with vehicle (hatched columns) or forskolin (10^{-5} M, solid columns). Each column represents mean±s.e. mean. + $P<0.05$ and ++ $P<0.01$ compared with vehicle group. ** $P<0.01$ compared with *S*-petasin at 0 M.

treatment were tested for homogeneity by a two-way analysis of variance, and the difference between specific means was tested for significance by Duncan's multiple-range test (16). In other cases, Student's *t*-test was employed. A difference between two means was considered statistically significant when $P<0.05$.

Results

Effects of S-petasin on Plasma Testosterone Concentration

The basal levels of plasma testosterone in vehicle and *S*-petasin challenged groups were 1.20±0.13 ng/ml (n=5) and 1.00±0.34 ng/ml (n=5), respectively. After 30 min injection of *S*-petasin, the concentration of plasma testosterone was reduced by 38.4% (vehicle group, 1.31±0.21 ng/ml, n=5, versus S-petasin group, 0.81±0.06 ng/ml, n=5, $P<0.05$).

Effects of S-petasin on Testosterone Secretion In Vitro

As compared with control group, the doses of S-petasin in the range of 4.3×10^{-7} ~ 4.3×10^{-5} M (0.14 ~ 14.4 μg/ml) caused a dose-dependent inhibition of testosterone release by rat testicular interstitial cells ($P<0.01$) (Fig. 2). Incubation of testicular interstitial cells with hCG (0.05 IU/ml) for 1 h increased the level of testosterone secretion ($P<0.01$). Combination of hCG with *S*-petasin (4.3×10^{-7} ~ 4.3×10^{-5} M) resulted in a significant inhibition of the hCG-stimulated release of testosterone ($P<0.01$). Administration of forskolin (10^{-5} M) significantly increased testosterone secretion ($P<0.01$) (Fig. 3). *S*-petasin (4.3×10^{-5} M) significantly decreased the stimulation of testosterone secretion induced by forskolin in testicular interstitial cells ($P<0.01$).

Effects of S-petasin on the Activity of 17-Ketosteroid Reductase

Administration of androstenedione (10^{-9} M), the testosterone biosynthesis precursors, significantly increased the production of testosterone in testicular interstitial cells ($P<0.01$) (Fig. 4). *S*-petasin at 4.3×10^{-6} M and 4.3×10^{-5} M decreased the production of testosterone in the presence of androstenedione in rat testicular interstitial cells ($P<0.05$ and $P<0.01$, respectively).

Discussion

Previous studies have shown that *S*-petasin inhibits the synthesis of peptido-leukotrienes (LTs) in mouse peritoneal macrophages cultures (2,3). This is the reason why *S*-petasin is clinically thought to be a non-steroidal anti-inflammatory agent *via* the reduction of LTs. The side effects of *S*-petasin in male patients had been ignored for a long time. The present study demonstrated that the acute injection of *S*-petasin inhibited the secretion of plasma testosterone. On the other hand, the administration of *S*-petasin in rat testicular interstitial cells diminished the basal, hCG-, forskolin-, and androstenedione-

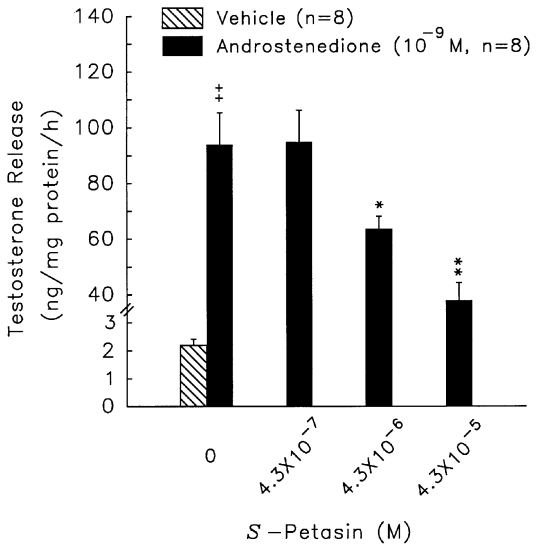

Fig. 4. Effects of *S*-petasin (0 ~ 4.3×10^{-5} M) on the testosterone release *in vitro* in rat testicular interstitial cells pretreated with vehicle (hatched column) or androstenedione (10^{-9} M, solid columns). Each column represents mean±s.e. mean. ++ $P<0.01$ compared with vehicle group. * $P<0.05$ and ** $P<0.01$ compared with *S*-petasin at 0 M.

stimulated secretion of testosterone *in vitro*.

It has been well known that hCG stimulates testosterone secretion both *in vivo* (10, 13, 19, 20) and *in vitro* (9, 15, 18, 19, 20) *via* an increase of the production of cyclic AMP (1, 12, 14, 19, 20). In the present study, we found that the stimulatory effects of hCG and forskolin on testosterone production *in vitro* were diminished by the administration of *S*-petasin. This reflected that *S*-petasin at 4.3×10^{-7} ~ 4.3×10^{-5} M might significantly inhibit the activity of adenylyl cyclase in rat testicular interstitial cells. The biosynthesis of steroid hormones by Leydig cells requires the sequential actions that convert cholesterol into various steroid classes (11). Androstenedione, one of steroidal precursor in steroidogenesis, is catalyzed by microsomal enzyme 17-KSR and produces testosterone. The inhibition of *S*-petasin to the stimulation of androstenedione demonstrated that S-petasin might directly act on 17-KSR to diminish the testosterone production.

Whether the secretions of gonadotropin releasing hormone by hypothalamus and luteinizing hormone by the anterior pituitary are decreased by the challenge of *S*-petasin is not known. *S*-petasin inhibits the mobilization of intracellular calcium ion (17) but the interaction between *S*-petasin and calcium-mobilizing stimuli in Leydig cell function has not been defined, either. It will be interesting to explore the role of *S*-petasin in modulating the control of hypothalamus-pituitary-testis axis and the relationship between *S*-petasin and calcium-mobilization in the future.

In summary, the present results demonstrated that *S*-petasin decreases testosterone production in part through diminishing the activities of adenylyl cyclase and 17-ketosteroid reductase.

Acknowledgments

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