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Effects of osthole on isolated guinea pig heart atria

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AIM: To study the effects of osthole (Ost) on the isolated guinea pig atria and the relationship between Ost effect and Ca^{2+}. **METHODS**: Contractions of left atria were induced by electric stimulations. The contractile amplitude of left atria pre- and post-treated with Ost was measured according to the cumulative concentration method, the drug being added at 15 min intervals, the pA_2 or pD_2' were calculated. It were measured that the effects of Ost to the positive staircase and to the post-rest potential enhancement. The contractile responses were recorded via an auto-equiolibration recording instrument. **RESULTS**: Ost 10—300 $\mu mol \cdot L^{-1}$ and Ver 0.1—30 $\mu mol \cdot L^{-1}$ decreased the contractile force and inhibited the isoprenaline-induced restoration of contractile response in the left atria rendered inexcitable by KCl 25 $mmol \cdot L^{-1}$. Ost and Ver antagonized the $CaCl_2$- and isoprenaline-induced positive inotropic response noncompetively, the pD_2' values to Ost were 4.41±0.13 and 4.90±0.15, to Ver were 6.53±0.22 and 6.91±0.17, respectively. Both of them inhibited the contraction of the left atrium and reversed the frequency-contraction response from positive to negative staircase in the higher dosage (500 and 1 $\mu mol \cdot L^{-1}$), but they showed only slight inhibitory effect on post-rest potentiation. **CONCLUSION**: Ost was similar to, but much less potent than Ver in inhibiting the isolated guinea pig atria.

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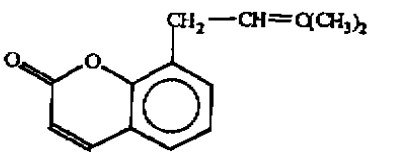
KEY WORDS osthole; coumarins; verapamil; calcium chloride; isoproterenol; heart atrium

Osthole (Ost) is a coumarin derivative extracted from fruits of *Cnidium monnieri* (L) Cusson. Its chemical name is 2H-1-benzopyran-2-one, 7-methoxy-8-(3-methyl-2) butenyl. Ost possesses hypotensive and antiarrhythmic action[1]. The contraction induced by norepinephrine, $CaCl_2$, or KCl is inhibited in the isolated rabbit aortic strips, and its mechanism was considered to be Ca^{2+} channel blockade[2]. We used the isolated atrium of guinea pig to investigate the effect of Ost, compared with a Ca^{2+} channel blocker verapamil (Ver).



Osthole

MATERIALS AND METHODS

Animals Guinea pigs ($n = 27$) of either sex weighing 338±s 26 g were provided by the Experimental Animal Center of our University.

Reagents Ost extracted by the Botanic Chemical Laboratory of Shaanxi Institute For Drug Control, purity is > 98 %, was dissolved in 1 % lactic acid, heated to 70 C to a concentration of 0.1 $mol \cdot L^{-1}$ before use, pH 7.2—7.4; Ver powder (Shanghai Institute of Biochemistry), was dissolved in 0.9 % normal saline to a concentration of 0.09 $mol \cdot L^{-1}$; propranolol (Pro) powder and isoprenaline (Iso) powder (Second Pharmaceutical Factory of Beijing) were dissolved in

0.9 % normal saline.

Methods The heart of guinea pig was placed in modified oxygenated Tris-Tyrode solution, then the left and right atria were suspended in 20 mL of the organ baths, at 34±0.5 C, pH 7.2—7.4. Contraction was recorded isometrically via a force-displacement transducer (made by Shanghai Medical University) connected to an auto-equilibration recording instrument (Model XWT-204). The right atria were not stimulated, the left ones were stimulated through field electrodes, on either side of the atria and the ones were taken out from the procedure-controlling stimulator (Model YC-2, the Instrument Factory of Chengdu, China). Rectangular current pulses of 150 % threshold voltage, at a frequency of 1 Hz for 3 ms. The atria were equilibrated under an optimal tension of 0.8—1.0 g for 1 h before specific experiment.

The contractile amplitude of left atria pre- and post-treated with Ost and Ver was measured according to the cumulative concentration method[3], the drugs being added at 15 min intervals, the pA_2 or pD_2' were calculated.

Modified Tyrode solution containing Tris and KCl 25 mmol · L^{-1} was used and the Na^+ concentration was reduced to 117 mmol · L^{-1} to maintain a constant osmotic pressure. The left atrium was given electric stimulation, which has stated above. Approximately 2 min later, the left atrial contraction stopped. After 5 min, Iso (3 μmol · L^{-1}) was added and electric stimulation with a rectangular pulse of 5 ms, 1 Hz, 200 % threshold intensity was applied, and the contractile amplitude of left atrium with / without drug was measured.

In the low-calcium (0.5 mmol · L^{-1}) modified Tris-Tyrode solution, the $CaCl_2$ dose-effect preference pre- or post-treated by drugs was measured. The Ca^{2+} concentration increased gradually in 2, 4, 8, 10 mmol · L^{-1} order, Ost and Ver were divided into low and high cincentrations. In the same solution, the $CaCl_2$ (3 mmol · L^{-1}) positive frequency effect pre- or post-treated by drugs to the right atrium rhyrhm was measured. In addition, Ost and Ver to the positive staircase of left atria and both to the post-rest potential enhancement of left atria were also measured[4].

Statistical analysis was self-contrast t test.

RESULTS

Contractile force of left atrium Both Ost (10—300 μmol · L^{-1}) and Ver (0.1—30 μmol · L^{-1}) showed concentration-dependent negative contractile force action, the maximal inhibiting rate that depressed the contractile force of left atria was 49±6 % ($n=9$, $P<0.01$) and 94±4 % ($n=6$, $P<0.01$), the concentration inhibiting 50 % muscle tension (IC_{50}) was 79 (70—88) and 0.28 (0.20—0.36) μmol · L^{-1}, respectively. The comparative intensity of Ost in inhibiting tension was 0.04 times higher than that of Ver.

Iso effect in left atrium Ost 100 μmol · L^{-1} and Ver 0.3 μmol · L^{-1} inhibited the concentration-dependent positive inotropic action of Iso to left atria, accompanying the reduction of maximal reaction. This demonstrated a noncompetitive antagonistic effect, the pD_2' value was 4.41±0.13 and 6.53±0.22, respectively. Pro 0.2 μmol · L^{-1} delayed the concentration-dependent positive inotropic effect of Iso without reduction of maximal reaction, showing a competitive antagonistic effect. The pA_2 value was 8.03±0.24 (Fig 1).

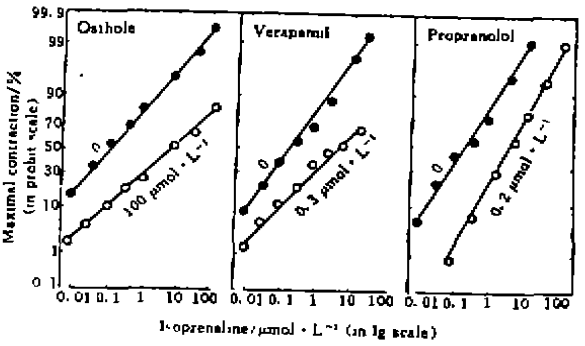


Fig 1. Effects of osthole, verapamil and propranolol on response of guinea pig left atria to isoprenaline. $n=7$, $\bar{x}\pm s$. (●) control.

High K^+ post-depolarization Iso recovering contractile force of left atrium Both Ost (10—100 μmol · L^{-1}) and Ver (0.1—30 μmol · L^{-1}) induced a concentration-dependent negative inotropic response and the maximal

inhibiting rate was 49±6 % (n = 7, P< 0.01) and 92±3 % (n = 6, P<0.01), and the IC_{50} was 38 (31 − 45) and 0.20 (0.15 −0.25) μmol · L^{-1}, respectively.

Left atrium Ca^{2+} effect Ost 10 and 100 μmol · L^{-1}, Ver 0.1 and 30 μmol · L^{-1} inhibited the concentration-dependent positive inotropic action of $CaCl_2$ to left atria, all of them accompanying the maximal response reduction, and showed noncompetitive inhibitory effects. The pD_2' value was 4.90±0.15 and 6.91±0.17, respectively (Fig 2).

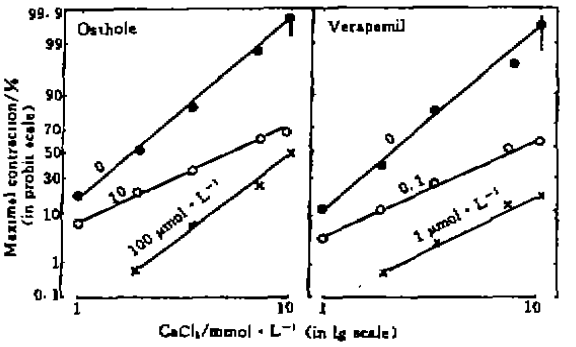


Fig 2. Effects of osthole and verapamil on response of guinea pig left atria to $CaCl_2$. n=6, $\bar{x}\pm s$. (●) **control.**

Right atrium rhythm induced by Ca^{2+} Ost 100 μmol · L^{-1} and Ver 0.05 μmol · L^{-1} reduced the spontaneous contractile frequency of right atrium by 93±2 % (n = 8, P< 0.01) and 106±4 % (n = 6, P<0.01), respectively.

Effects of Ost and Ver on positive staircase phenomena of left atrium and post-rest potential enhancement Ost 500 μmol · L^{-1} and Ver 1 μmol · L^{-1} reversed the frequency-contraction response of left atria from positive to negative staircase (Fig 3). This inhibitory effect was more obvious at a higher frequency-dependent negative inotropic effect. Ost (100 −500 μmol · L^{-1}) reduced the post-rest potential enhancement of left atria by 88±13 % (n = 8, P>0.05) and 75±10 % (n = 6, P<0.01), respectively and Ver (0.3−1 μmol · L^{-1}) reduced it by 91±8 % (n = 6, P> 0.05) and 63±5 % (n = 6, P<0.01), respectively. The results using Ver corresponded with those of previous reports[5, 6].

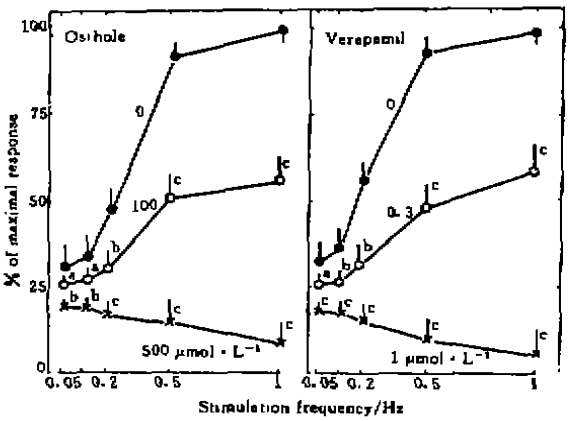


Fig 3. Effects of osthole and verapamil on force-frequency relationship in guinea pig left atria. n=7, $\bar{x}\pm s$. ^bP>0.05. ^bP<0.05, ^cP<0.01 *vs* **control.**

DISCUSSION

Our experiment demonstrated that Ost reduced the contractility of isolated guinea pig left atrium, it showed a stronger inhibitory effect on left atrium contraction induced by high K^+ depolarization and Iso recovering the transmembrane calcium influx. Ost also inhibited right atrium positive frequency and left atrium positive inotropic response caused by Ca^{2+}. The type of action was similar to that of Ver, but the effect of Ost was much weaker than that of the latter. The result suggested that Ost might have the effect of inhibiting the transmembrane calcium influx.

Comparing the effect of Ost on the dose-effect curve of left atrium positive inotropic response induced by Iso with that of Ver and Pro, it was illustrated that Ost has the characteristics of calcium channel blocker, but not those of β-receptor blocker.

Positive staircase phenomena implied that accompanying the increase of stimulated frequency, the influx of Ca^{2+} into the cell increased[7]. It also may be related to sarcolemmal calcium leaking at rest[8, 9]. Post-rest potentiation enhancement was mainly caused by increasing the release of Ca^{2+} from the reservoir inside the cells[10]. Ost inhibited the positive staircase phenomena in a concentration- and frequency-dependent manner and reversed the phenomena at a higher frequency. It showed only slight inhibitory effects on post-rest potentiation at a higher concentration. All these results suggested that Ost exhibited the important characteristics of calcium channel blocker through inhibiting Ca^{2+} influx from outside the cell, but showed a mild effect on Ca^{2+} release from calcium resevoir inside the cell. As to the nature of the effects of Ost on antagonizing Ca^{2+}, it needs to be further studied.

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蛇床子素对离体豚鼠心房的作用

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A **目的**: 研究蛇床子素对离体豚鼠心房的作用及与Ca^{2+}的关系. **方法**: 钙拮抗剂 Ver 对照, 电刺激左房收缩, 累积浓度法测定给 Ost 前后左房收缩幅度, 给药间隔15 min, 计算 pA_2 或 pD_2'; 观察 Ost 对左房收缩正阶梯、静息后增强作用。收缩反应由 XWT-204台式平衡记录仪描记. **结果**: Ost、Ver 产生浓度依赖性负性肌力作用, 抑制高 K^+ Iso 恢复的左房收缩; 非竞争性拮抗 Iso、$CaCl_2$正性肌力作用, 相应 pD_2' 值分别为4.41±0.13和6.53±0.22, 4.90±0.15和6.91±0.17; 抑制正阶梯, 大剂量使之翻转, 对静息后增强作用弱. **结论**: Ost 对心房抑制作用与 Ver 相似, 但强度较后者弱, 可能抑制胞外钙内流.

关键词 蛇床子素; 香豆素类; 维拉帕米; 氯化钙; 异丙肾上腺; 心房