组胺刺激 30 min,与单纯应用组胺的比较,K_f和 J_v值明显降低和 σ 值的明显升高,差异明显($P < 0.01$)。说明当归补血汤对组胺造成的 EC单层对蛋白质和大分子物质的通透性的增加具有较强的抑制作用。

表 2 当归补血汤对含组胺的 5 g/L白蛋白 Hanks灌流液引起的 EC单层通透性增加的作用 ($\bar{x} \pm s$)

组 别	n	K_f (μL/min· cm²· kPa)	J_v (μL/min· cm²· kPa)	σ
对照组	5	4.62± 0.53	11.16± 1.11	0.52± 0.02
组胺组	7	6.27± 0.82*	14.89± 2.18*	0.26± 0.04**
当归补血汤组	4	4.53± 0.78△△	11.38± 1.81△	0.38± 0.03△

与对照组比较: * $P < 0.05$ ** $P < 0.01$; 与组胺组比较: △ $P < 0.05$ △△ $P < 0.01$

在内皮细胞膜上介导的炎症介质受体有组胺、缓激肽、血小板激活因子(PAF)和细胞因子等。当这些受体被激活后,引起内皮细胞的收缩,导致细胞旁路的开放或跨细胞穿透的膜孔径增宽,随之使血管内皮通透性增加[7]。其通透性增加机制涉及到细胞外 [Ca^{2+}] 的内流和细胞内 [Ca^{2+}] 的释放;信号转导系统的激活,包括 G蛋白和磷脂酶 C(PLC)信号、蛋白激酶(PKC)和酪氨酸激酶(TK)的激活、以及肌球蛋白轻链(MLC)的磷酸化和去磷酸化等[8,9]。近年来,MLC的磷酸化和去磷酸化的研究倍受重视。细胞外 [Ca^{2+}] 的内流和细胞内 [Ca^{2+}] 的释放以及 MLC的去磷酸化能够诱导肌动-肌球蛋白间相互作用,中心张力增加,引起细胞骨架的变化和细胞间裂隙形成[10]。应用 [Ca^{2+}] 内流阻断剂和外源性 cAMP能够抑制炎症介质所致的内皮通透性增加[9]。

本实验采用内皮单层加压灌注装置,精确地定量 Starling方程中的各个参数 K_f、J_v和 σ。在此,K_f和 J_v值反映的是内皮单层对液体和小分子物质的通透性,σ 值反映的是内皮单层对大分子物质的通透性。内皮细胞单层经含组胺的 hanks液和含组胺的 5 g/L白蛋白 hanks液灌流后,对液体和小分子物质的通透性以及对蛋白质和大分子物质的通透性均明显增高。内皮单层经组胺刺激后,应用当归补血汤能够逆转因组胺引起的内皮单层的通秀性增加,说明当归补血汤对组胺造成的 EC单层通透性增加具有改善作用。根据本实验的结果,还不能确定当归补血汤改善内皮单层的通透性的作用机制,进一步的工作将着重研究当归补血汤在抗炎症作用中的细胞和分子机制,特别是对信号转导系统的影响。

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Studies on the Calcium Antagonist Action of Arctigenin

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Abstract The calcium antagonist action of arctigenin (ACT) was studied in order to verify the effect of *Fructus Arctii* for the relieve of exterior syndrome. Muscular contraction of isolated rat trachea, colon, pulmonary artery and thoracic aorta induced by KCl, that of guinea pig trachea induced by $CaCl_2$, before and after the addition of ACT were assessed and their contraction-response curves drawn and PD 2 calculated according to Scott. The inhibition rate of two-phase contraction of guinea pig trachea induced by acetylcholine chloride (Ach) in comparison with verapamil (VER) was calculated. Results of the study showed that ACT could non-compatitively antagonize the muscular contraction of the test specimens with

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PD$_2$ of 4.01, 5.11, 5.98 and 6.05 respectively. Similar to VER, ACT could non-competitively antagonize the isolated guinea pig trachea with PD$_2$ of 4.04 and 5.62 respectively. Both of them could inhibit the first phase contraction induced by Ach with inhibition rates of 66.14% and 81.42% respectively. It was concluded that ACT, as the active constituent of *Fructus Arctii*, relaxed smooth muscle contraction by blocking the potential dependant Ca^{2+} channel and the internal release of Ca^{2+}.

Key words arctigenin smooth muscle contraction calcium antagnist

摘 要 对牛蒡苷元(ACT)的钙拮抗作用进行探讨,为确认 ACT是牛蒡子解表功能的有效成分提供实验依据。描记给 ACT前后,各标本对 KCl或 $CaCl_2$诱发收缩的量效曲线,按 Scott法计算 PD$_2$;测定给 ACT后,标本对乙酰胆碱(Ach)诱发的两相收缩的抑制百分率。ACT对离体大鼠气管、结肠、肺动脉、胸主动脉平滑肌由 KCl引起的收缩产生非竞争性拮抗作用,其 PD$_2$分别为 4.01, 5.11, 5.98, 6.05; ACT和维拉帕米(Verapamil)相似,对离体豚鼠气管平滑肌由 CaCl引起的收缩产生非竞争性拮抗作用,其 PD$_2$分别为 4.04, 5.62;对 Ach诱发的两相收缩只明显抑制第一时相收缩,抑制率分别为 66.14%, 81.42%。ACT对平滑肌的松弛作用可能是由于阻滞电压依赖性钙通道和内钙释放所致;ACT是牛蒡子解表功能的有效成分。

关键词 牛蒡苷元 平滑肌收缩 钙拮抗剂

Fructus Arctii, the dried fruit of *Arctium lappa* L. (*Compositae*) is a traditional Chinese medicine pungent in flavor but cooling in effect. It was used as an antipyretic for the relieve of syndrome of internal heat. It acts to dispel exterior wind while clear away internal heat; by promoting the dispersing function of the lung for the complete eruption of the conjunctiva and air passage, thus removing toxic materials and benefits the pharynx. ACT, a lignan isolated from *Fructus Arctii* has been reported in literatures to possess the therapeutic effect of lowering elevated blood pressure by blocking the Ca^{2+} channel[1]. Thus, it is justifiable to make a deeper inquiry into the Ca^{2+} antagonist effect of ACT.

1 Material and Method

1.1 Material: ACT was isolated and purified from *Fructus Arctii* to a purity of 99.8% by Dr. Xu Zhaohui and Prof. Yang Songsong of Department of Phytochemistry of this College. It was dissolved in water to concentrations of 8.06×10^{-4}, 2.69×10^{-3} and 8.06×10^{-3} mol/L with the addition of 60, 200 and 200 ml/L of absolute alcohol respectively.

VER, product of Shanghai Tianfeng Pharmaceutical Factory.

Ach, product of Shanghai Third Reagent Factory.

Kreb's solution; Ca^{2+} free Kreb's solution and high potassium Ca^{2+} free Kreb's solution were prepared after the modified Polster's physiological salt solutions[2].

Wistar rat and guinea pig of either sex weighing (250± 50) g and (300± 50) g respectively were supplied by Liaoning Vivisection Center, College of TCM.

LWA-20 Force displacement transducer, product of Shanghai Medicine Electronic Instrument Plant.

XWT-204 Potentiometric recorder, product of Shanghai Dahua Instrument and Meter Plant.

1.2 Method: The isolated smooth muscle specimens, prepared to their respective suitable length (trachea, from the lower end of larynx to its eminence were sectioned into rings of 4~ 5 mm segments; colon, fasted for 48 h, sectioned into 1 cm segments; pulmonary artery and thoracic aorta, sectioned into 3~ 4 mm segments), were suspended in a bath filled with 10 mL Kreb's solution maintained at a temperature of (37± 1) ℃ and aerated with a mixture of 95% O_2 and 5% CO_2. The specimens were connected to the force displacement transducer and loaded with an initial tension of 1 g. The solutions were renewed every 20 min to maintain freshness. After equilibrium, the test drugs were added cumulatively, the added amount should not exceed 2% of the total volume of the nutrient bath. On each addition, the contraction of

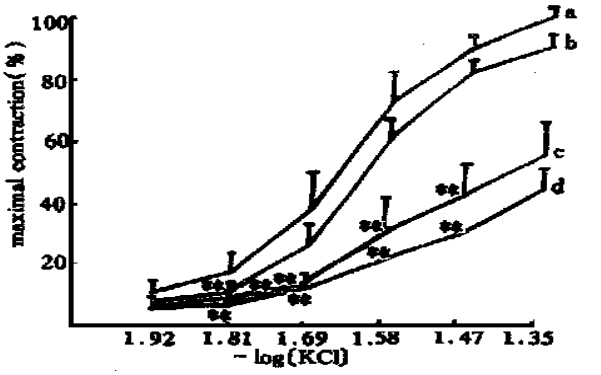
the specimen was recorded with the potentiometric recorder. The terminal pH was not significantly changed when the drugs were added. Same amount of alcohol used as blank control showed no significant effect on the contraction of the specimens. Data were expressed in $\bar{x} \pm s$ and statistically compared by t-test. PD_2 values were calculated according to Scott. The maximal response of the control was recorded as 100%, and the concentration-response curves (CRC) were constructed accordingly.

2 Results

2.1 Effects of ACT on the contraction of 4 smooth muscle specimens induced by KCl Contractions induced by a final concentration of 30 mmol/L of KCl was used as the control to assess the activities of the specimens. Cumulative addition of KCl to a final concentration(mmol/L) of 12 ~ 44 for trachea, 9.0 ~ 24.6 for colon, 6.0 ~ 39.3 for pulmonary artery and thoracic aorta were used as auto-control for their respective CRC. The final concentrations (mol/L) of ACT added were 3.226×10^{-6}, 1.075×10^{-5} and 3.226×10^{-5} respectively. They were incubated for 10 min and the addition of KCl was repeated as described above for CRC data which were shown in Fig. 1 ~ 4. It could be seen that ACT at the ultimate concentrations mentioned above could shift the KCl CRC to the right, depicting its non-competitive Ca^{2+} antagonist effects. The calculated PD_2 were 4.01, 5.11, 5.98 and 6.05 respectively.

2.2 Effects of ACT on $CaCl_2$ induced CRC of guinea pig trachea ACT at the ultimate concentrations of 3.226×10^{-6}, 1.075×10^{-5} and 3.226×10^{-5} mol/L and VER at 5×10^{-7}, 1×10^{-6} and 2×10^{-6} mol/L also showed non-competitve antagonist effect (Fig. 5 and 6). The calculated PD_2 were 4.04 and 5.62 respectively.

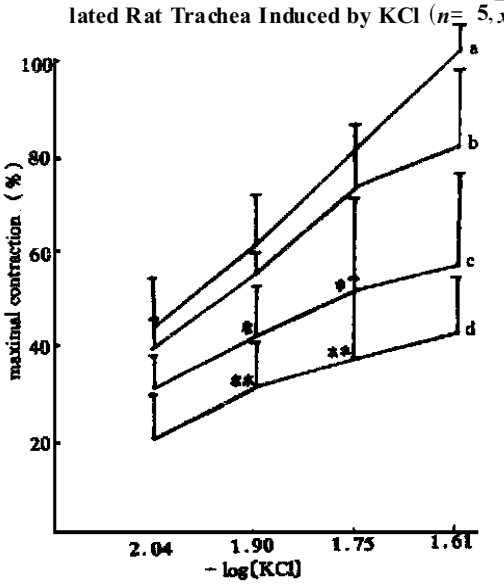
2.3 Effects of ACT on two-phase contraction induced by Ach ACT at concentrations of 1.29×10^{-4}, 1.72×10^{-4} mol/L and VER at 7.5×10^{-6}, and 1.0×10^{-5} mol/L showed inhibition of the first-phase contraction but no significant effect on second-phase contraction (Fig. 7). Their rates of inhibition were 49.13% and 66.14% by ACT, and 57.46% and 81.42% by VER respectively.



a-control; b-3.226×10^{-6} mol/L; c-1.075×10^{-5} mol/L; d-3.226×10^{-5} mol/L

compared to the control * $P < 0.05$ ** $P < 0.01$

Fig. 1 Effects of Arctigenin on the Contraction of Isolated Rat Trachea Induced by KCl ($n = 5, \bar{x} \pm s$)



a-control; b-3.226×10^{-6} mol/L; c-1.075×10^{-5} mol/L; d-3.226×10^{-5} mol/L

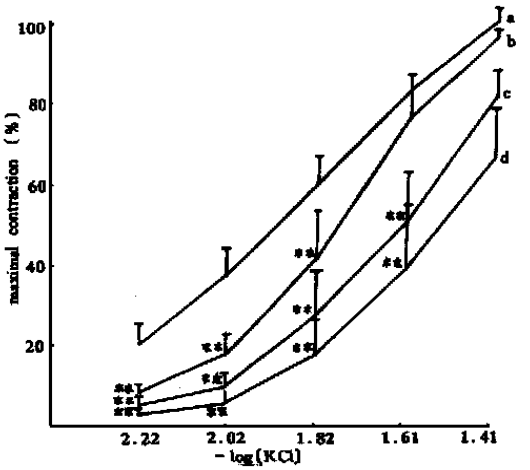
compared to the control * $P < 0.05$ ** $P < 0.01$

Fig. 2 Effects of Arctigenin on the Contraction of Isolated Rat Colon Induced by KCl ($n = 5, \bar{x} \pm s$)

3 Discussion

High K^+ caused smooth muscle contraction by opening the potential dependent channels (PDC) on the cellular membrane during depolarization to promote extracellular Ca^{2+} influx[3]. The action of ACT, similar to VER is to block the PDC and inhibit extracellular Ca^{2+} influx. Thus, it suppresses intracellular calcium mobilization rather than calcium entry via receptor operated channels (ROC)[4].

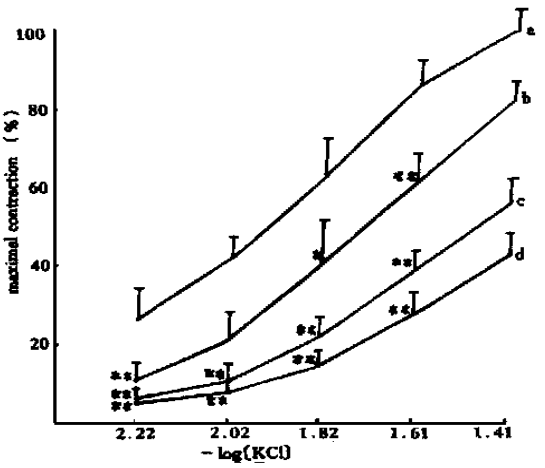
Ca^{2+} antagonists have been extensitvely used



a-control; b-3.226× 10^{-6} mol/L; c-1.075× 10^{-5} mol/L; d-3.226× 10^{-5} mol/L

compared to the control * $P < 0.05$ ** $P < 0.01$

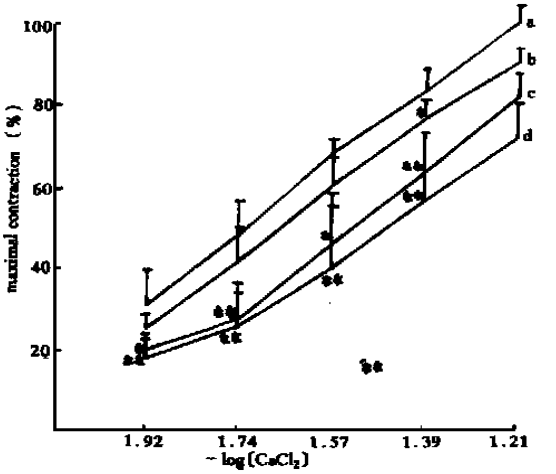
Fig. 3 Effects of Arctigenin on the Contraction of Isolated Rat Pulmonary Artery Induced by KCl (n= 5, $\bar{x} \pm s$)



a-control; b-3.226× 10^{-6} mol/L; c-1.075× 10^{-5} mol/L; d-3.226× 10^{-5} mol/L

compared to the control * $P < 0.05$ ** $P < 0.01$

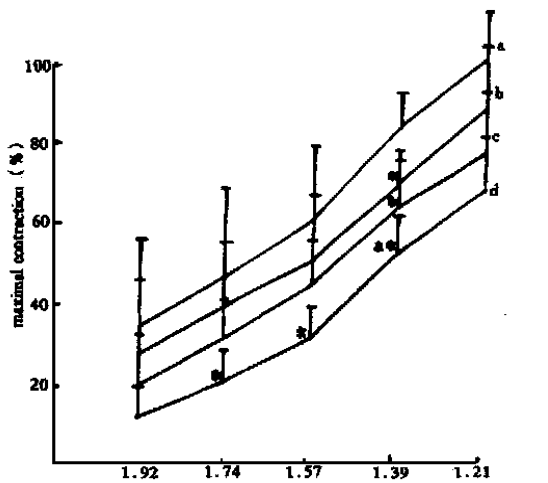
Fig. 4 Effects of Arctigenin on the Contraction of Isolated Rat Thoracic Aorta Induced by KCl (n= 5, $\bar{x} \pm s$)



a-control; b-3.226× 10^{-6} mol/L; c-1.075× 10^{-5} mol/L; d-3.226× 10^{-5} mol/L

compared to the control * $P < 0.05$ ** $P < 0.01$

Fig. 5 Effects of Arctigenin on the Contraction of Isolated Guinea Pig Trachea Induced by $CaCl_2$ (n= 5, $\bar{x} \pm s$)



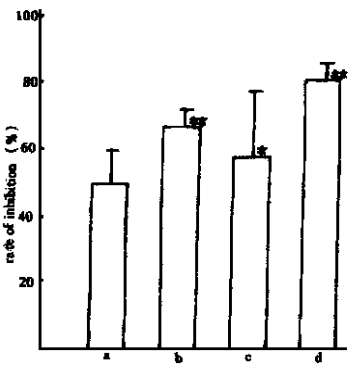
a-control; b-5× 10^{-7} mol/L; c-1× 10^{-6} mol/L; d-2× 10^{-6} mol/L

compared to the control * $P < 0.05$ ** $P < 0.01$

Fig. 6 Effects of VER on the Contraction of Isolated Guinea Pig Trachea Induced by $CaCl_2$ (n= 5, $\bar{x} \pm s$)

to treat cardiovascular diseases. Recently, it was reported that bronchial asthma was also related with abnormal Ca^{2+} movement. Ca^{2+} antagonists can relax bronchial smooth muscle and block the production of inflammatory mediators of the air passage at different stages of asthmatic attacks. Moreover, this kind of drugs may show a synergetic effect with other antiasthmatics and reduce the dose of hormone used by hormone-dependent patients[5]. As a new type of Ca^{2+} antagonist, ACT merits to be developed as a drug of choice for treatment of both cardiovascular diseases and bronchial asthma.

ACT could relax thoracic aorta, increase the circulating blood volume of organs, such as skin and relax the smooth muscle of pulmonary artery and trachea. Numerous literatures and our own experience showed that ACT could resist the attack



a–ACT 1.29× 10^{-4} mol/L; b–ACT 1.72× 10^{-4}; c–VER 7.5× 10^{-6} mol/L; d–VER 1.0× 10^{-5} mol/L

compared to the control: * $P < 0.05$ ** $P < 0.01$

Fig. 7 Effects of Arctigenin on the Intracellular Calcium-Dependent Contraction (n= 5, $\bar{x} \pm s$)

of some pathogens, such as bacteria, fungi and virus due to its action to increase immunological functions. In traditional Chinese medical concept, pulmonary functions and intestinal symptoms represent the exterior and interior manifestations of diseases. The relaxation of colon may facilitated bowel movement to relieve gastrointestinal cold. Result of the present study showed that ACT, the mainactive principle of *Fructus Arctii*, can relieve the exterior syndrome with simultaneous improvement of intestinal peristalsis. Determination of the ACT content in *Fructus Arctii* is a suitable and feasible quality control criterion to assess the quality of the drug.

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钩藤中不同成分降压作用的差异△

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摘 要 目的: 比较钩藤中提取的异钩藤碱、钩藤碱、钩藤总碱及非生物碱部分的降压作用。方法: 大鼠麻醉后经颈总动脉插管记录外周血压和经股静脉微量输注实验用药。结果: 实验表明钩藤中 4种成分的降压强度为异钩藤碱 (42.0%)> 钩藤碱 (32.1%)> 钩藤总碱 (21.3%)> 钩藤非生物碱 (12.4%)。结论: 提示钩藤中主要降压成分为异钩藤碱和钩藤碱。

关键词 钩藤 钩藤碱 异钩藤碱 钩藤总碱 钩藤非生物碱 外周血压

Different Hypotentive Effects of Various Active Constituents Isolated from *Uncaria rhynchophylla*

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Abstract Hypotensive effects of rhynchophylline, isorhynchophylline, total alkaloid and non-alkaloid fraction isolated from *Uncaria rhynchophylla* (Miq.) Jacks. were compared. Change in peripheral blood pressure was recorded by inserting a catheter into the right common carotid artery of anaethetized rat, while each of the four hypotensive constituents was injected individually into the femoral vein by a microinfusion pump. Results of the findings showed that the four constituents in *U. rhynchophylla* displayed different hypotensive potency in the order of isorhynchophylline [lowering of mean arterial pressure (MAP) by 42.0%] > rhynchophylline (lowering of MAP by 32.1%) > total alkaloid (lowering of MAP by 21.3%) > non-alkaloid fraction (lowering of MAP by 12.4%). It was concluded that isorhynchophylline and rhynchophylline were the main hypotensive constituents in *U. rhynchophylla*.

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