BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 May; 16 (3): 231—234

Inhibitory effect of tetrandrine on C fiber activation-induced contractions in trachea and bronchus of guinea pig[1]

FANG Li-Ben, YANG Qiu-Huo, BIAN Ru-Lian
(*Department of Pharmacology, Zhejiang Medical University, Hangzhou 310006, China*)

AIM: To study the inhibitory effects of tetrandrine (Tet) on an activation of the sensory nervous C fibers in isolated trachea and bronchus of guinea pig. **METHOD**: The electric field stimulation induced a phase I contraction of the preparations, which was due to an activation of the C fifers. The effects of Tet on phase I contraction were analyzed. **RESULTS**: Tet 0.3—30 $\mu mol \cdot L^{-1}$ inhibited phase I contractions in a concentration-dependent manner. That phase I contractions inhibited by Tet 1 $\mu mol \cdot L^{-1}$ were 40 ±38 % and 75±22 % of control in tracheae and bronchi respectively. After pretreatment with chlorphenamine or atropine phase I contraction were still reduced by Tet 1 $\mu mol \cdot L^{-1}$, inhibitory rates being 70±16 % and 64±16 % of control, respectively. The contractile responses of the preparations to exogenous substance P, however, were unaffected by treatment with Tet 1 $\mu mol \cdot L^{-1}$. **CONCLUSION**: Tet 1 $\mu mol \cdot L^{-1}$ inhibits phase I contraction, related to the inhibition of local release of neuropeptides from C fibers of guinea pig airway.

KEY WORDS tetrandrine; substance P; electric stimulation; trachea; bronchi; nerve fibers

The electric field stimulation (EFS) evokes the release of transmitters from nerve endings in the isolated trachea or bronchus *in vitro*, inducing a rapid contraction (phase I contraction followed by a long-standing contraction (phase II contraction) of the smooth muscle[1,2]. The phase I contraction of airway smooth muscles is induced by the release of neuropeptides, such as substance P (SP) and neurokinins, from the capsaicin-sensitive sensory nerves (ie. the C fibers)[3]. The neuropeptides have both direct and indirect constrictive effects on the smooth muscle of airway, stimulating the release of histamine from mast cells (in trachea) or of acetylcholine from cholinergic endings (in bronchus) in guinea pig[7]. Tetrandrine (Tet), a calcium channel blocker extracted from Chinese herb (*Stephania tetrandra* S Moore), relaxes the smooth muscle of blood vessels directly[4], and inhibits the contractile actions of smooth muscle of the airway as well as the release of histamine from the mast cell[5,6]. A drug that inhibits the release of neuropeptides from sensory nerves or prevents the activation of airway C fibers may be beneficial to the treatment of asthma[8,9]. In this experiment we want to know whether an inhibitory effect of Tet is involved in the activation of C fibers in guinea pig airway.

MATERIALS AND METHODS

Hartley guinea pigs (n=18) of either sex, weighing 360±70 g, supplied by the Experimental Animal Center of Zhejiang Medical University, were stunned and exsanguinated. The isolated tracheae and the main bronchi suspended between 2 platinum ring electrodes in a 10-mL organ bath containing Krebs-Henseleit solution were connected to a force-displacement transducer for measurement of isometric tension[1]. The tissues were equilibrated for 60 min under an initial ten-

[1] Project supported by Natural Science Foundation of Zhejiang Province, China. № 391043.
Received 1993-12-07 Accepted 1994-08-30

sion of 0.5 g, and washed with fresh solution every 20 min. Propranolol (3 μmol·L^{-1}) and indometacin (2—3 μmol·L^{-1}) were added in the physiological solution.

EFS (48 V, 15 Hz, 0.8 ms pulse width, trains of 15 s duration) were applied at an interval of 30 min.

Tet was obtained from Jinhua Pharmaceutical Factory, Zhejiang; SP and indometacin, from Sigma.

Phase I contraction tension was expressed as mg ($\bar{x}\pm s$). Differences between means were analyzed by paired t test and group t test.

The experiment included: 1) Escalating concentrations of Tet inhibited phase I contration; 2) Tet <3 μmol·L^{-1} effected phase II contractions with pretreatment of H_1-receptor or M-receptor antagonists; 3) Tet <3 μmol·L^{-1} effected the airway contractions induced by escalating concentrations of SP.

RESULTS

When the tracheae or bronchi were stimulated by EFS, phase I and phase II contractions occurred. Escalating concentrations of Tet (0.3—30 μmol·L^{-1}) inhibited phase II contraction of tracheae ($P<0.01$) and bronchi ($P<0.01$) in a concentration-dependent manner (Tab 1).

After treating the trachea with an H_1-receptor antagonist, chlorphenamine (Chl) 1 μmol·L^{-1}, or treating the bronchus with an M-receptor antagonist, atropine (Atr) 1 μmol·L^{-1}, Tet 1 μmol·L^{-1} still inhibited phase II contractions of the airway smooth muscle (Tab 2).

Tab 2. C fiber stimulation-induced contraction of tracheae (+ chlorphenamine 1 μmol·L^{-1}) or bronchi (+ atropine 1 μmol·L^{-1}), after tetrandrine 1 μmol·L^{-1}. $^bP<0.05$, $^cP<0.01$ *vs* control.

	n	Control mg	Treatment with tetrandrine mg	% of control
Tracheae	8	69±42	50±34^c	70±16^c
Bronchi	5	57±28	37±20^b	64±16^c

Exogenous SP 0.3—3 μmol·L^{-1} induced contractions of the trachea and bronchus preparations obviously. Pretreatment with Tet 1 μmol·L^{-1} did not effect the contraction induced by exogenous SP ($P>0.05$, Tab 3).

Tab 3. Substance P induced contraction (mg) of tracheae or bronchi, which were not affected by tetrandrine 1 μmol·L^{-1}. $^aP>0.05$ *vs* control.

	n	Substance P/μmol·L^{-1} 0.3	1.0	3.0
Tracheae				
Control	4	137±35	208±68	224±75
Tet	4	115±16^a	192±16^a	223±14^a
Bronchi				
Control	6	76±20	138±23	165±26
Tet	6	75±8^a	143±8^a	177±12^a

Tab 1. Phase II contraction by EFS after tetrandrine 0.3—30 μmol·L^{-1}. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

	Tetrandrine/μmol·L^{-1} 0	0.3	1.0	3.0	10	30
Tracheae						
n	16	13	11	13	13	13
mg	48±17	29±13^c	21±23^c	12±12^c	7±12^c	5±9^c
%	—	63±21	40±38	24±23	17±31	11±24
Bronchi						
n	11	9	11	11	11	8
mg	51±16	48±14^a	37±14^b	35±16^b	31±16^c	17±15^c
%	—	87±16	75±22	73±31	62±37	32±29

DISCUSSION

While the preparations of guinea pig airway were stimulated by EFS, the local release of neuropeptides (eg. SP) from the C-fibers induced a long-standing contraction of the smooth muscles (phase II contraction). The responses were similar to those in previous reports[1-3]. Tet inhibited phase II contraction of the preparations in a concentration-dependent manner. It could be a directly inhibitory effect on the smooth muscle, because Tet relaxed the smooth muscle of blood vessels and trachea in dog (>10 $\mu mol \cdot L^{-1}$) or in guinea pig (>3 $\mu mol \cdot L^{-1}$) in a higher concentration[4,6]. Our results showed that there was an obviously inhibitory effect on phase II contraction when the cocentractions of Tet were <3 $\mu mol \cdot L^{-1}$. It suggested that the inhibitory effect of Tet 0.3—1 $\mu mol \cdot L^{-1}$ on the airway smooth muscle was not a directly inhibitory one. It could be that other inhibitory mechanisms were involved.

Our previous experiments suggested that histamine released from mast cells in trachea, or acetylcholine in bronchus increased phase II contraction of the preparation respectively[7]. However, phase II contractions of the tracheal preparation pretreated with an H_1-receptor antagonist and the bronchial preparation pretreated with an M-receptor antagonist were still inhibited by Tet. The concentration of Tet used (1 $\mu mol \cdot L^{-1}$) was much lower than that reported (250 $\mu mol \cdot L^{-1}$) for inhibiting the release of histamine from mast cells[5]. It was presumed that inhibitory effects of Tet 1 $\mu mol \cdot L^{-1}$ on phase II contraciton did not include a release of histamine or acetylcholine.

SP is a neuropeptide existing in the C fiber of guinea pig airway. Exogenous SP induced a contraction of tracheal and bronchial preparations in a concentration-dependent manner. The lower-concentration of Tet I $\mu mol \cdot L^{-1}$ did not show inhibitory effects on the contraction induced by the exogenous SP. It was further shown that the effect of Tet (I $\mu mol \cdot L^{-1}$) inhibited phase II contractions is not a direct one on the smooth muscle contraction.

These came to the conclusion that the lower-concentration of Tet (1 $\mu mol \cdot L^{-1}$) is effective against phase II contraction. the results suggested that one of the actions of Tet is related to the inhibition of local release of neuropeptides from the C fibers in guinea pig airway.

REFERENCES

1 Fang LB, Yang QH, Bian RL. Airway contraction of guinea-pigs induced by the activation of the sensory nerve C-fibers *in vitro*.
J Zhejiang Med Univ 1993; **22**: 193—5.

2 Ellis JL, Undem VJ. Non-adrenergic, non-cholinergic contractions in the electrically field stimulated guinea-pig trachea. Br J Pharmacol 1990; **101**: 875—80.

3 Lundberg JM. Peptide and classical transmitter mechanisms in the autonomic nervous system.
Arch Int Pharmacodyn Ther 1990; **303**: 9—19.

4 Hu WS, Pang XB, Wang Y, Hu CJ, Lu FH. Mode of action of tetrandrine on the smooth muscle of blood vessels. J Tradit chin Med 1982; **23**: 227—30.

5 Li W, Zhou HL, Yang QH, Bian RL. Inhibition on function of rat peritoneal mast cells by tetrandrine.
Acta Pharmacol Sin 1987; **8**: 450—3.

6 Zhou HL, Xie QM, Bian RL. Effects of tetrandrine on ^{45}Ca influx in tracheal smooth muscles.
Acta Pharm Sin 1987; **22**: 405—8.

7 Fang LB, Yang QH, Bian RL. Mediator difference in contracitons between trachea and bronchus of guinea pig induced by stimulation of C-fibers *in vitro*.
Acta Pharmacol Sin 1994; **15** (5): 436—8.

8 Barnes PJ. Effect of nedocromil sodium on airway sensory nerves. J Allergy Clin Immunol 1993; **92**: 182—6.

9 Lou YP. Regulation of neuropeptide release from pulmonary capsaicin-sensitive afferents in relation to bronchoconstriction.
Acta Physiol Scand 1993; **149** Suppl 612: 1—88.

粉防己碱抑制豚鼠离体气道 C 神经纤维兴奋

231-234

引起的收缩

方理本、杨秋文、卞如濂
(浙江医科大学药理学教研室，杭州310006，中国)

目的：研究粉防己碱(Tet)对豚鼠离体气管/支气管的感觉神经C纤维兴奋的抑制作用. **方法**：记录电场刺激所致的C纤维兴奋所产生的标本收缩(phase II)张力，了解Tet的作用. **结果**：Tet 0.3—30 μmol·L^{-1}抑制phase II收缩，在气管/支气管上，Tet 1 μmol·L^{-1}的抑制率分别是：40±38 %和75±22 %；用氯苯那敏或阿托品作用后，Tet 1 μmol·L^{-1}的抑制率分别是70±16 %和64±16 %；Tet不抑制外源性P物质引起的标本收缩. **结论**：Tet 1 μmol·L^{-1}抑制豚鼠离体气道收缩的机理与其抑制感觉神经C纤维兴奋释放神经肽的作用有关.

关键词 粉防己碱；P物质；电刺激；气管；支气管；神经纤维

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 May; 16 (3): 234—238

Effect of schisanhenol on function and surface shape of rat neutrophils[1]

ZHANG Ke-Jian, LIN Tong-Jun, QIN Ling, LIU Geng-Tao (*Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, China*)

AIM: To study the regulatory effect of Schisanhenol (Sal) on function of rat neutrophils. **RESULTS**: It was found that Sal (1, 10, and 100 μmol·L^{-1}) inhibited neutrophil activities such as chemotaxis, phagocytosis, and superoxide anion production *in vitro* in a concentration-dependent manner. Changes of surface morphology of neutrophils were observed by scanning electron microscopy, showing that the ruffles and pseudopods on neutrophil surface increased under the stimulation by chemotactic peptide *N*-formyl-Met-Leu-Phe (FMLP). When pretreated with Sal 100 μmol·L^{-1}, the ruffles and pseudopods disappeared and the surface became smooth. Sal 100 μmol·L^{-1} decreased the cytosolic calcium concentration of neutrophils and increased the intracellular cAMP level. **CONCLUSION**: These data suggested that Sal could inhibit the function of rat neutrophils through affecting the cytosolic free calcium and cAMP level besides its antioxidant activity.

KEY WORDS schisanhenol; neutrophils; chemotaxis; phagocytosis; calcium; cyclic AMP; cyclic GMP

Schisanhenol (Sal) isolated from *Schizandrae rubriflora* has many pharmacological actions, such as antioxidant activity, induction of liver microsomal cytochrome p-450, protective action against CCl_4-, alcohol-, and *D*-galactosamine-induced liver injury[1—3]. In the *D*-galactosamine-induced liver injury, there was infiltration of neutrophils and macrophages in the liver tissue[4]. The parallel occurrence of both neutrophil degradation and

[1]Project supported by the National Natural Science Foundation of China (№ 39170232) and HUO Ying-Dong Education Foundation.
Received 1993-05-31 Accepted 1994-07-04