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Drug responses in antigen–induced contractions of tracheal strips of ovalbumin–sensitized guinea pigs[1]

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ABSTRACT Guinea pig trachea strips were isolated after 21 d of ovalbumin–sensitization. Challenging the preparations with antigen induced a double–phase contractile response (DPCR) which consisted of a rapid contraction (RC) and a tonic contraction (TC). The effects of tetrodotoxin (TTX), atropine, hexamethonium, diphenhydramine and a substance P (SP) antagonist on the DPCR were observed. DPCR was insensitive to atropine or hexamethonium at 1 μmol / L. Diphenhydramine 2 μmol / L abolished RC, and inhibited TC by 48.0 ± 5.6%. TTX 1 μmol / L and lidocaine 2 μmol / L almost completely abolished TC and reduced RC by 49.6 ± 6.7% and 44.6 ± 8.4%, respectively. The substance P antagonist, ([*D*–Arg[1], *D*–2,4–diCl$_2$–Phe[5], Asp[6], *D*–Trp[7,9], Nle[11])– SP 10 μmol / L, inhibited RC by 49.9 ± 6.1% and TC by 90.7 ± 9.3%. These results suggest that SP sensory nerve fibres in respiratory tract play an important role in the pathogenesis of DPCR, especially in that of TC.

KEY WORDS substance P; trachea; ovalbumin

Substance P (SP) sensory nerve fibres are widely present in mammalian respiratory tract[1]. Stimulation of these fibres or administration of exogenous SP induces contraction of respiratory smooth muscle and increase in both vascular permeability and excretion of the glands in the respiratory tract[2,3]. Overexcitation of these sensory fibres by environmental stimuli might be involved in the pathogenesis of asthma[4]. But no conclusive evidence supports the involvement of these mechanisms in the antigen–induced broncheospasm. In this paper, antigen–induced contractile responses of the ovalbumin–sensitized guinea pig trachea (OSGPT) *in vitro* were used as a model to observe the effects of diphenhydramine, atropine, TTX, hexamethonium and a substance P antagonist on the contractile responses to demonstrate whether or not SP sensory nerve fibres take part in the pathogenesis of asthma.

MATERIALS AND METHODS

Guinea pigs, ♂, weighing 265 ± SD 48 g were sensitized by 12.5 % ovalbumin saline (0.4 ml ip ± 0.4 ml sc) and control groups with saline alone. After 21–28 d, guinea pigs were stunned and exsanguinated. The trachea was removed and prepared as spiral strips[5]. Two strips were made from one trachea, of which each contained 2 smooth muscle segments. They were divided into control and experimental groups. The strips were suspended in a 5 ml organ bath containing 95% O_2 and 5% CO_2, 37 ℃ Krebs solution[6], and then stretched with an initial tension of 1 g. After a stabilization period of over 60 min, the preparations were challenged by 50 μl of 0.025% ovalbumin saline. The changes in tension of the preparations were recorded by a force–displacement transducer and a recorder (Shanghai Dahua Apparatus Factory, modle No. LM–14–224). The antigen was added 10 min after addition of the drugs. Only one challenge was performed on each preparation. The results were expressed as $\bar{x}$ ± SD. Satistical comparison were performed by *t* test. SP antagonist [*D*–

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Arg–Pro–Lys–Pro–*D*–diCl_2–Phe–Asp–*D*–Trp–Phe–*D*–Trp–Leu–Nle–SP] (powder, synthetized by the solid phase method on a Beckman model 990 Peptide Synthetizer and purified by HPLC) supplied by Department of Fine Engineering Chemicals of East China Chemical University. Diphenhydramine, atropine, hexamethonium and lidocaine were produced by Tian Fang Pharmaceutical Factory, Shanghai. Tetrodotoxin (TTX) was supplied by Hebei Aquatic Product Institute.

RESULTS

Acetylcholine (2 μmol / L) induced a contractile response (261 ± 43 mg) in unsensitized guinea pig trachea, but ovalbumin (0.025%, 50 μl) had no remarkable effect on the preparation (n= 9). Contractions of OSGPT were not evoked by bovine serum albumin (10 μg), but were induced by an ovalbumin challenge (0.025%, 50 μl). Ovalbumin challenge of OSGPT caused a double–phase contractile response (DPCR) consisting of a rapid contraction (RC) and a tonic contraction (TC). After addition of the antigen, the tension of OSGPT increased rapidly (348 ± 97 mg, n=18), and decreased quickly. This contractile response was named RC, which was followed by a slow recovery of the tension, arriving at the mean peak value of 389 ± 88 mg (n=18), and well maintained for over 15 min. This was termed as TC, as shown in Fig 1A. The latent time (LT) of DPCR which was defined as the period from addition of the antigen to the beginning of RC was 58 ± 11 s (n=18).

The effects of the drugs on DPCR were shown in Fig 1 and Tab 1. The DPCR showed resistance to atropine and hexamethnium bromide (n=6). Diphenhydramine abolished RC and inhibited TC by 4.80 ± 5.6% (n=7). The drug prolonged the LT about 5 times of the controls, as shown in Fig 1A and Tab 1. TTX and lidocaine reduced RC by 49.6 ± 6.7% and 44.6 ± 8.4%

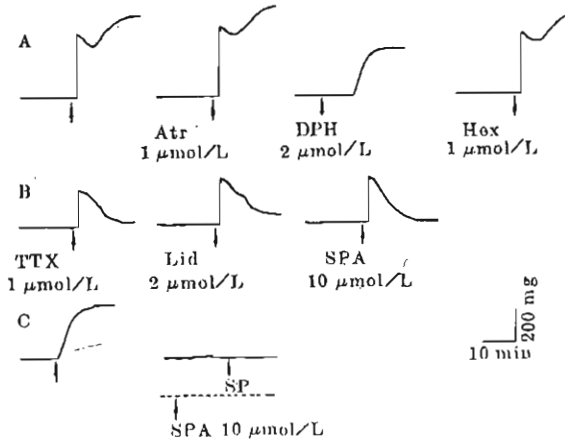

Fig 1. Contractions of guinea pig trachea strips sensitized by ovalbumin 0.025%, 50 μl(A,B) and substance P 2 μmol / L (C), respectively. Atr = Atropine; DPH = Diphenhydramine; Hex = Hexamethonium; TTX = Tetrodotoxin; Lic = Lidocaine; SPA = SP antagonist.

respectively, inhibited TC by 92.5 ± 2.1 % and 77.0 ± 4.6% respectively and no significantly effect on LT. The curves of DPCR was changed into RC in reduced amplitude (n=6) as shown in Fig 1B. The SP antagonist, (D–Arg[1], D–2,4–diCl_2–Phe[5], Asp[6], D–Trp[7,9], Nle[11])– SP, of which the pA_2 value obtained on guinea pig trachea was 6.64 ± 0.27[(7)], inhibited RC by 49.9 ± 6.1% and TC by 90.7 ± 9.3 %, but did not significantly influence LT (n=9). Compared the remains of the tension in the presence of the SP antagonist to that of controls, the difference was highly significant (P<0.01) as shown in Tab 1. Exogenous SP (2 μmol / L) induced a slow contraction (151 ± 54 mg) of OSGPT and well maintained at the level for over 10 min (n=6). The effect of SP was blocked completely by the SP antagonist (10 μmol / L) as shown in Fig 1C.

DISCUSSION

Antigen challenge of OSGPT *in vitro* induced tracheospasm characterised with TC and RC. TTX and lidocaine abolish the overwhelming part of TC, but hexamethonium and atropine have no significant effect on it,

Tab 1. Effects of drugs on the double-phase contractile responses of sensitized guinea pig trachea induced by the challenge of ovalbumin (0.025%, 50μmol / L). $\bar{x} \pm SD$. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* control.

Drug (μmol / L)	n	Latent time(s)		Rapid contraction (mg)		Tonic contraction(mg)	
		Control	Test	Control	Test	Control	Test
Atropine	(1) 6	58 ± 13	60 ± 10*	343 ± 90	329 ± 98*	369 ± 88	361 ± 84*
Hexamethonium	(1) 6	57 ± 8	59 ± 13*	320 ± 56	309 ± 79*	342 ± 77	397 ± 70*
Diphenhydramine	(2) 7	61 ± 11	300 ± 58***	348 ± 90	3 ± 5***	381 ± 76	189 ± 56***
SP antagonist	(10) 9	59 ± 8	58 ± 6*	441 ± 93	221 ± 88***	495 ± 174	46 ± 28***
Lidocaine	(2) 6	59 ± 16	57 ± 13*	325 ± 52	195 ± 71***	370 ± 62	85 ± 41***
TTX	(1) 6	56 ± 11	61 ± 10*	371 ± 14	187 ± 51***	388 ± 70	29 ± 8***

suggesting that TC is closely related to the effect of nervous reflex of non-cholinergic postganglionic fibres. Moreover, the SP antagonist inhibits TC by 90.7 ± 9.3%. These results show that the nerve fibres involved in the reflex might be SP sensory nerve fibres distributed in respiratory tract. Although according to the significant inhibitory effect of diphenhydramine on RC, the RC might be primarily induced by histamine, neuronal reflex also involves in it. The mechanism might be like this: antigen-antibody reaction stimulates SP-sensory nerve fibres directly or indirectly and induces the release of SP, the later, in addition to directly evoking a slow contraction, promotes the release of histamine from mast cells[8], which takes part in the pathogenesis of RC. In this way neuronal reflex mechanism attends the rapid contraction of DPCR. So TTX, lidocaine and the SP antagonist can also inhibit RC significantly.

These data strongly support the theory suggested by Barnes[4] concerning that SP sensory nerve fibres in respiratory tract involed in the pathogenesis of asthma and might introduce a clue to look for new effecient antiasthma drugs.

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药物在抗原引起卵白蛋白致敏豚鼠气管条收缩中的反应

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提要 用卵白蛋白致敏 21 d 后的豚鼠气管制备气管条. 抗原攻击此标本引起双相收缩反应, 即快速收缩相和痉挛收缩相. 观察河豚毒素, 阿托品, 六甲双铵, 苯海拉明和 P 物质拮抗剂对此双相收缩反应的作用. 此反应对阿托品和六甲双铵不敏感. 苯海拉明消除此双相反应中的快速收缩相, 抑制痉挛收缩相 48.0 ± 5.6%. 河豚毒素和利多卡因几乎完全消除痉挛收缩相, 分别抑制快速收缩相 49.6 ± 6.7%和 44.6 ± 8.4%. 10 μmol / L 的 P 物质拮抗剂, (D-Arg[1]-D-2,4-di-Cl-Phe[5], Asp[6], Nle[11])-SP 10 μmol / L, 抑制快速收缩相 49.6 ± 6.1%和痉挛收缩相 90.7 ± 9.3%. 实验表明, 呼吸道内的 P 物质感觉神经纤维参与了此双相收缩反应. 尤其是痉挛收缩相.

关键词 P 物质; 气管; 卵白蛋白