

## Drug Responses of Canine Trachea, Bronchus and Bronchiole

YOSHIKAZU YAMATAKE, SUMIKO SASAGAWA, and SAIZO YANAURA

*Department of Pharmacology, Hoshi College of Pharmacy<sup>1)</sup>*

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Effects of acetylcholine, pilocarpine, histamine, 5-hydroxytryptamine and nicotine on the isolated canine trachea, bronchus and bronchiole were investigated. In addition, Schultz-Dale test to *Ascaris suum* antigen on these airway smooth muscle preparations was attempted. Acetylcholine had a strong contractile effect on the trachea, bronchus and bronchiole, whereas large concentrations of histamine and nicotine were necessary to contract these preparations. Histamine was suggested to produce a stronger constriction of the peripheral airways like bronchioles. Pilocarpine showed a selective and remarkable contraction of bronchus. Schultz-Dale phenomenon to *Ascaris* antigen failed to be demonstrated on the airway preparations.

**Keywords**—trachea; bronchus; bronchiole; biogenic amines; Schultz-Dale reaction; *Ascaris suum*

Although the isolated tracheal preparations have been much used for studying airway smooth muscle,<sup>2)</sup> responses of bronchi and bronchioles are more significant in bronchial asthma. There is, however, no reports in which drug responsiveness of trachea, bronchus and bronchiole was compared with. Schultz-Dale phenomenon of canine airway smooth muscles has not been also documented.

In the present study, we investigated contractility of these three portions of the canine respiratory tract responding to a few amines by using isolated preparations, and attempted Schultz-Dale test on these preparations.

### Materials and methods

**Preparation**—Male mongrel dogs weighing 7–13 kg were anesthetized with sodium pentobarbital 30 mg/kg *i.v.* and exsanguinated. The trachea was exposed and the 2nd to 4th (from larynx) tracheal rings were excised. The cartilaginous part of a tracheal ring of 5 mm in width was cut so that two pieces of cartilage remained attached to both ends of the muscle strip. The segment was about 15 mm in length. Strips of bronchus and bronchiole were prepared as follows. After exsanguination, left thoracotomy was made at the 5th intercostal space. The left lung with the left main bronchus was excised, and bronchus ring at the level of the 2nd or 3rd bifurcation from the trachea was cut open and a 5 mm wide and about 15 mm long segment was made. Bronchiole (o.d. about 1 mm) at the level of the 6th bifurcation from the trachea was sectioned out from the left lobus diaphragmaticus. The bronchiole was spirally cut and a 1 mm wide and about 15 mm long segment was made.

Each preparation made as described above at the experimental day was placed in a tissue-organ bath containing Tyrode solution with a final volume of 20 ml. The experimental solution was continuously oxygenated with a 95% O<sub>2</sub>–5% CO<sub>2</sub> mixture and maintained at a constant temperature of 37°. Isometric contractions were recorded with a force-displacement transducer on a polygraph (RM-85, Nihon Kohden Co., Ltd.). The trachea, bronchus and bronchiole were equilibrated for 30 min under a resting tension of 1.0, 0.4 and 0.2 g, respectively.

**Schultz-Dale Test**—For an antigen, *Ascaris suum* antigen prepared following the method described previously<sup>3)</sup> was used. Dogs naturally sensitive to *Ascaris* antigen were selected with skin test, in which animals that showed significant blueings in concentrations of below 10<sup>-6</sup> g protein/ml of the antigen solu-

1) Location: Ebara, Shinagawa-ku, Tokyo 142, Japan.

2) a) J.C. Castillo and E.J. de Beer, *J. Pharmacol. Exp. Ther.*, **90**, 104 (1947); b) A. Akcasu, *J. Pharm. Pharmacol.*, **4**, 671 (1952); c) K. Takagi, I. Takayanagi and K. Fujie, *Chem. Pharm. Bull.* (Tokyo), **6**, 716 (1958).

3) Y. Yamatake, S. Sasagawa, S. Yanaura and N. Kobayashi, *Japan. J. Pharmacol.*, **27**, 285 (1977).

tion were estimated to be positive. Trachea, bronchus and bronchiole of the dog with positive skin reaction were used for Schultz-Dale test. *Ascaris* antigen was given into the organ bath by single injection.

**Drugs**—The drugs used were acetylcholine chloride (ACh, Ovisot, Daiich Seiyaku), pilocarpine hydrochloride (Hoei Yakko), 5-hydroxytryptamine creatinine sulfate (5-HT, Tokyo Kasei), histamine dihydrochloride (Wako Pure Chemicals) and nicotine bitartrate (Tokyo Kasei). All drug solutions except for nicotine were added by single injection. All concentrations are expressed as the salt and as final concentrations in g/ml.

## Results

None of the preparations used in the experiment showed spontaneous movement. Effects of ACh, pilocarpine, 5-HT, histamine and nicotine on the trachea are shown in Fig. 1. Among the contractile actions of these agents, only that of nicotine was transient (phasic). ACh, pilocarpine and 5-HT possessed a strong contractile effect on the trachea, the threshold concentrations being  $10^{-10}$ – $10^{-9}$  g/ml, while histamine and nicotine had a low activity on the trachea, the threshold being  $10^{-8}$ – $10^{-7}$  and  $10^{-5}$  g/ml, respectively.

In contrast with trachea, bronchus was especially sensitive to pilocarpine (Fig. 2), the activity being above 300 fold stronger than that of ACh. On the other hand, the sensitivity of bronchus to ACh was about 100 fold lower than that of trachea, although histamine, 5-HT and nicotine had similar threshold concentrations both in the bronchus and trachea.

Responses of bronchiole to histamine was more prominent than those of trachea and bronchus in spite that tension produced was relatively small. The maximal effect of pilocarpine was low in the bronchiole (Fig. 3).

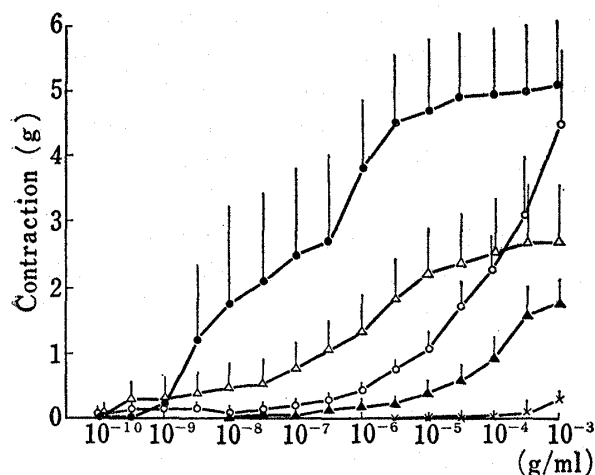


Fig. 2. log-dose Response Curves for Several Amines on the Canine Isolated Bronchus

Each point represents the mean with S.E. for five to six experiments. —○—, acetylcholine; —●—, pilocarpine; —▲—, histamine; —△—, 5-hydroxytryptamine; —×—, nicotine.

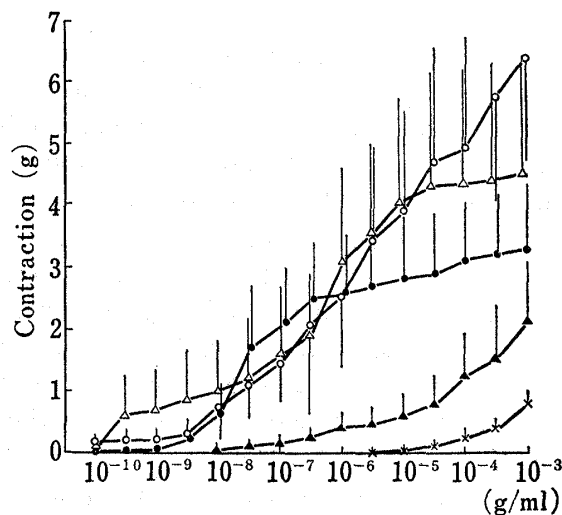


Fig. 1. log-dose Response Curves for Several Amines on the Canine Isolated Trachea

Each point represents the mean with S.E. for five to six experiments. —○—, acetylcholine; —●—, pilocarpine; —▲—, histamine; —△—, 5-hydroxytryptamine; —×—, nicotine.

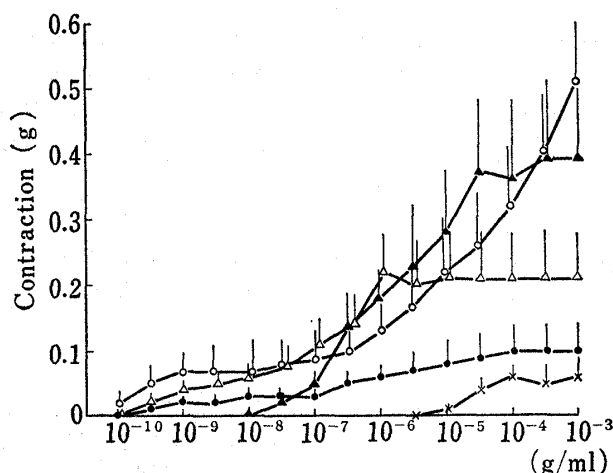


Fig. 3. log-dose Curves for Several Amines on the Canine Isolated Brochiole

Each point represents the mean with S.E. for five to six experiments. —○—, acetylcholine; —●—, pilocarpine; —▲—, histamine; —△—, 5-hydroxytryptamine; —×—, nicotine.

Time for attaining to a plateau of response after drug administration was short for ACh and nicotine, and relatively long for pilocarpine, 5-HT and histamine in all the three portions of respiratory tract.

Contraction of the trachea, bronchus and bronchiole after challenge with *Ascaris* antigen in concentrations of  $10^{-5}$ ,  $3 \times 10^{-5}$  and  $10^{-4}$  g protein/ml failed to be demonstrated in all cases of twelve experiments for the former two preparations and six ones for the latter.

### Discussion

Electrical stimulation of the cervical vagus nerve has been shown, by the use of a rapid lung freezing method in dogs,<sup>4)</sup> to produce constrictions of the whole airways from trachea to bronchioles having inner diameters of 1—2 mm. The present finding that ACh caused an intense contractile effect on all the tracheal, bronchial and bronchiolar preparations of the dog suggests an important role of vagal activities on the tones of whole airway smooth muscles.

Akcasu<sup>2b)</sup> reported that the minimal concentrations of ACh necessary to produce contraction of isolated tracheal preparations of guinea pig, human, dog, cat, rabbit and rat were  $10^{-7}$ ,  $10^{-5}$ ,  $10^{-9}$ ,  $10^{-8}$ ,  $10^{-6}$  and  $10^{-6}$  g/ml, respectively, and concluded that the trachea of the dog is extremely sensitive to ACh. The minimal concentrations of ACh to contract the canine trachea, bronchus and bronchiole were  $10^{-10}$ — $10^{-9}$ ,  $10^{-8}$ — $10^{-7}$  and  $10^{-10}$  g/ml, respectively in the present experiment. The data on the canine trachea are, therefore, approximately consistent with that of Akcasu.

Woolcock, *et al.*<sup>5)</sup> demonstrated that bronchi having inner diameters of 3 to 8 mm provide 50% of the total airway resistance. This does not mean that the bronchi are not always most sensitive to drugs including ACh, among various portions of the airways, since the bronchus, at least, lobar bronchus had a similar or a rather less sensitivity to ACh, histamine, 5-HT and nicotine except for pilocarpine than the trachea and bronchiole.

The contractile potencies of histamine were much less than those of ACh in the trachea and bronchus. ACh and histamine are reportedly equipotent for an increase in pulmonary resistance *in vivo* in dogs.<sup>6)</sup> Bronchoconstriction induced by histamine has been found to be, in a most part, mediated *via* vagal reflex in *in vivo* experiments.<sup>3,7)</sup> The present result that only extremely high concentrations of histamine could contract the isolated airway smooth muscles such as trachea and bronchus would also suggest that bronchoconstriction induced by small doses of histamine *in vivo* may be produced *via* cholinergic systems and that induced by high doses of histamine *via* both cholinergic systems and a direct action on the smooth muscles. Furthermore, the finding that the bronchiole was much more sensitive to histamine than the trachea and bronchus is consistent with the histological result by Colebatch, *et al.*<sup>8)</sup> that histamine injected into the right heart *in vivo* in cats showed constriction of respiratory bronchioles and alveolar ducts, while ACh constricted chiefly conducting airways, that is trachea and bronchi.

The bronchoconstriction observed in pulmonary embolism in dogs is attributed to 5-HT released from platelets.<sup>9)</sup> The sensitivities in terms of threshold concentration of the trachea, bronchus and bronchiole to 5-HT were approximately the same as those of ACh, though the maximal contractions were low.

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