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A possible site of action of nicotine in the bronchial smooth muscle preparation of guinea-pig

I. TAKAYANAGI*, Y. KIZAWA, *Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, Funabashi, Chiba 274, Japan*

The bronchioles are innervated by excitatory cholinergic parasympathetic and inhibitory adrenergic sympathetic nerve fibres. Ganglion cells exist in the lung distributed along the branches of the bronchial tree (Macklin 1929). Actions of nicotine on the guinea-pig isolated trachea are well known (Hawkins & Paton 1958; Chiou & Long 1969; Jones et al 1980) but only Hawkins & Paton (1958) have reported on the action of nicotine on the isolated bronchial muscle of guinea-pig.

Male guinea-pigs, 350 to 400 g, were killed by a blow on the head and main bronchi isolated and cut helically. The preparations (2 × 30 mm) were suspended in a 20 ml organ bath filled with a physiological solution (NaCl 118, KCl 4·72, $CaCl_2$ 2·56, $MgSO_4.7H_2O$ 0·16, KH_2PO_4 1·20, $NaHCO_3$ 25·0 and dextrose 10·0 mM) gassed with carbogen and kept at 32 °C. Responses to drugs were recorded isometrically under a tension of 0·5 g. In some experiments, two platinum electrodes (2 mm × 35 mm) were placed 5 mm apart and field stimulation of the bronchial preparations was carried out by passing a rectangular pulse of 0·5 ms duration, supramaximal voltage and a frequency of 10 Hz between the two electrodes for 10 s. The experiments were started after the preparations had developed their spontaneous tone for 60 min. All agonists were applied to the preparation at intervals of 60 min. The concentration of cyclic (c) GMP was measured by the methods of Steiner et al (1972) to estimate the effects of nicotine and acetylcholine on a tissue concentration of cGMP. Two pieces of bronchus were prepared. One was used for measuring the control concentration of cGMP and the other for any change after exposure to a drug. Protein concentration was measured by the method of Lowry et al (1951), with bovine serum albumin as the standard.

Contractile response to nicotine was reproducible under the conditions used. Nicotine (10^{-6}–10^{-3} M) contracted the bronchial smooth muscle concentration-dependently (Fig. 1). The maximum response to nicotine was 38·5 ± 4·0% (mean ± s.e.m. of 6 experiments) of that to acetylcholine. No inhibitory response to nicotine (10^{-6}–10^{-3} M) was observed in any preparation used; all were greatly relaxed by 10^{-4} M papaverine (Fig. 1). In the following experiments, nicotine 10^{-4} M and the equieffective acetylcholine 10^{-4} M were used as agonists. Ganglion blockers, hexamethonium (10^{-5} M) and pentolinium (10^{-6} M) at concentrations that were enough to inhibit the concentration action curve of nicotine in the guinea-pig ileum (Van Rossum 1962), considerably reduced the contractile response to nicotine. But this was not influenced by 5 min treatment with atropine (10^{-6} M) which almost inhibited the responses to acetylcholine 10^{-4} M (Table 1). Fifteen min treatment of the bronchus with tetrodotoxin (3 × 10^{-6} M) also was without any effect on the response to nicotine. Furthermore, SX-284 (2-(1,2-benzisoxazol-3-yl)-3-[2-(2-piperdinoethoxy)phenyl]acrylonitrile) (3 × 10^{-7} M), which inhibits acetylcholine release from the parasympathetic nerve specifically (Takayanagi et al 1982), did not influence the nicotine-induced contraction. Field stimulation induced a contractile response

Table 1. Effects of some drugs on the contractile responses to nicotine, acetylcholine and field stimulation. Each value is presented as a mean ± s.e.m. of 6 experiments. ( ): incubation time. SX-284 is an inhibitor of acetylcholine release from parasympathetic nerves (Takayanagi et al 1982).

Treatment	% of contraction
Nicotine, 10^{-4} M	100·0
+ hexamethonium, 10^{-5} M (5 min)	32·7 ± 2·8*
+ pentolinium, 10^{-6} M (5 min)	17·0 ± 3·5*
+ atropine, 10^{-6} M (5 min)	95·0 ± 8·1
+ tetrodotoxin, 3 × 10^{-6} M (15 min)	99·7 ± 11·4
+ diphenhydramine, 10^{-6} M (5 min)	94·8 ± 7·6
+ indomethacin, 10^{-6} M (30 min)	112·3 ± 15·6
+ SX-284, 3 × 10^{-7} M (15 min)	93·6 ± 8·3
+ physostigmine, 10^{-6} M (30 min)	103·6 ± 7·6
Acetylcholine, 10^{-4} M	100·0
+ atropine, 10^{-6} M (5 min)	12·4 ± 4·9*
+ physostigmine, 10^{-6} M (30 min)	229·3 ± 19·3*
Field stimulation	100·0
+ tetrodotoxin, 3 × 10^{-6} M (15 min)	15·7 ± 4·8*
+ physostigmine, 10^{-6} M (30 min)	147·7 ± 5·9*

* Significant difference from 100% at $P < 0.05$.

* Correspondence.

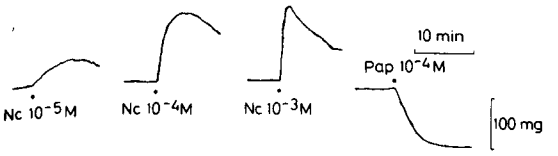

FIG. 1. Responses of the guinea-pig bronchial preparation to nicotine and to papaverine. Nc: nicotine, Pap: papaverine. Horizontal and longitudinal bars: 10 min and 100 mg.

which was about 40% of that to nicotine 10^{-4} M. Thirty min treatment of the preparation with physostigmine (10^{-6} M), which potentiated the contractile responses to field stimulation and to acetylcholine, was without any effect on the response to nicotine. Furthermore, nicotine-induced contractions were not influenced by 30 min treatment with indomethacin (10^{-6} M) (Table 1).

In separate experiments we estimated the cGMP content in the bronchial smooth muscle 4 min after application of acetylcholine or nicotine (10^{-4} M). Four min were needed for the bronchial preparation to contract to maximum amplitude. The tissue concentration of cGMP was significantly increased by acetylcholine but not by nicotine (Fig. 2).

Hawkins & Paton (1958) reported that, when responses to nicotine were isotonically recorded, these had three components all of which were greatly reduced or abolished by hexamethonium: the first component was a rapid transient contraction abolished by atropine, the second contractile component appeared not to be cholinergic, and the third component was the dilator response. When the response to nicotine was recorded isometrically in our study, only one type of contractile response to nicotine was observed, although the bronchial preparation was much relaxed by papaverine (Fig. 1). The contractile response was inhibited by the ganglion blockers but not influenced by atropine, diphenhydramine or physostigmine (Table 1). Therefore, the contractile response to nicotine in our study seems to be similar to the second component of response reported by Hawkins & Paton (1958). The results with indomethacin (Table 1) suggest that the contractile response to nicotine is not due to a release of prostaglandins, nor was it influenced by SX-284, an inhibitor of acetylcholine release from parasympathetic nerve (Takayanagi et al 1982). The tissue concentration of cGMP was increased by acetylcholine but not by

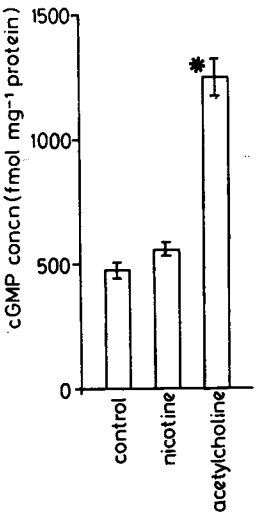

FIG. 2. Effects of nicotine and acetylcholine on the tissue concentration cGMP. Nicotine 10^{-4} M, acetylcholine 10^{-4} M. Each value is presented as a mean (column) with s.e.m. (bar) of 10 experiments. *: significant difference from the control value at $P < 0.05$.

nicotine (Fig. 2). These facts suggest that in this study nicotine did not bring about its effect by stimulation of cholinergic ganglion cells. Further evidence that tetrodotoxin did not influence the contractile response of the bronchial preparation to nicotine suggests that a possible site of action of nicotine is on the smooth muscle cells and not on the nerve cells. However, we could not rule out a contribution by chemical mediators released by nicotine in the contractile mechanisms.

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