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THE ACTION OF DRUGS ON ISOLATED MAMMALIAN BRONCHIAL MUSCLE

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Recently the pharmacological preparation consisting of a chain of tracheal or bronchial rings has been much used (Castillo and de Beer, 1947). Results based on relaxation of tone suggest that the relative potencies of bronchodilators in guinea-pig tracheal chains are of the same order as those found in human bronchial preparations (Hawkins and Schild, 1951). We have studied the response to histamine and acetylcholine of the circular muscle of similar preparations made from several mammals. The relative dilator activities of sympathomimetic amines and aminophylline on the contracted tracheal muscle have been compared with those found on contracted human bronchial preparations.

METHODS

Tracheae of cats, dogs, rabbits, guinea-pigs, and rats were removed immediately after death. All tissues were carefully dissected and cut into rings which were tied together in chains with loops of cotton. In most experiments the cartilage in each ring was cut so that only the mucosa and smooth muscle band were left joining each ring together. The mucosa was then removed to allow of greater freedom of movement of the muscle. One to six rings (total length 2 to 3 cm.) were suspended in an isolated organ bath (volume 15 ml.) at 37° C. containing oxygenated Tyrode solution. Human bronchi (internal diameter 5–10 mm.) were secured from the post-mortem room as soon as possible after death; the delay was as long as 10 hours on one occasion, but the other specimens were obtained not more than 7 hours after death. The responses were recorded with a very light writing lever (magnification $\times 10$) on a slowly revolving kymograph. Histamine acid phosphate and acetylcholine chloride were left in contact with the preparation for one to three minutes. The preparation was washed twice after the addition of each drug and usually returned to baseline within five minutes. Antagonist or bronchodilator drugs were introduced into the bath usually 30 seconds before the histamine or acetylcholine doses. Most preparations responded well for at least 6 hours, and many were used after 24 hours' storage at 4° C.

The drugs used were synthetic *l*-adrenaline, *l*-noradrenaline *d*-bitartrate monohydrate, *dl-iso*propylnoradrenaline hydrochloride (isoprenaline), and *l*-ephedrine hydrochloride. All solutions were made up in 0.01 N-HCl, and dilutions were made with distilled water. Aminophylline (Burroughs Wellcome) was also studied. Drug concentrations are given in terms of the active base.

RESULTS

Action of Acetylcholine

Acetylcholine causes contraction of all the chains, and recovery to the baseline is rapid when the bath fluid is changed. Effective concentrations are: dog, 10^{-8}; cat, 10^{-7}; rabbit, 10^{-6}; rat, guinea-pig, and human, 10^{-5}.

For the comparative assay of bronchodilator drugs, the dose of adrenaline necessary to reduce by 50% the contraction produced by acetylcholine was taken as unity. Two dose levels of acetylcholine and antagonist were usually used.

The results for all the bronchodilators studied are shown in Table I. Each value is the geometric mean of at least six separate experiments. The results on the human bronchi showed considerable variation (see Table I for range), but this was much less in the animal experiments. Isoprenaline in every species tested was more active than adrenaline in reducing the acetylcholine-induced spasm (Fig. 1). Noradrenaline possesses about one-hundredth the activity of adrenaline on human tissue (Fig. 2), but it is equipotent in the rabbit and cat. The rabbit preparation is relatively insensitive to sympathomimetic amines, the adrenaline concentration for antagonism being as high as 10^{-6}, whereas in the other preparations the corresponding concentration for antagonism is 10^{-8} to 10^{-7}.

Action of Histamine

When added to the bath, histamine causes contraction of preparations from some species. Recovery may be slow, however, when it is washed out of the human preparation (average concentra-

TABLE I

EQUIACTIVE DOSES OF BRONCHODILATOR DRUGS

Stimulants are histamine (Hist.) and acetylcholine (Ach.). Figures in brackets indicate range of individual results of human experiments.

Preparation:	Human Bronchial Chain			Tracheal Rings						
				Guinea-pig		Dog		Rat	Rabbit	Cat
Constrictor:	Hist.	Ach.	None*	Hist.	Ach.	Hist.	Ach.	Ach.	Ach.	Ach.
dl-Isoprenaline..	0.5 (0.25–1)	0.4 (0.25–1)	0.19	0.25	0.23	0.23	0.25	0.13	0.25	0.11
l-Adrenaline ..	1	1	1	1	1	1	1	1	1	1
l-Noradrenaline	100 (50–200)	75 (20–200)	23(*dl*)	5	5	2	2	2	1	1
l-Ephedrine ..	1,700 (500–2,000)	1,200 (500–2,000)	710	470	490	500	500	400	290	280
Aminophylline..	500 (250–1,000)	2,300 (1,250–7,500)	1,900	1,800	10,300	1,600	10,100	7,300	10,000	15,400

* Hawkins and Schild (1951).

tion 10^{-5}). Histamine (10^{-6}) stimulates the guinea-pig and dog preparations and the relative potencies of the bronchodilators (except aminophylline) are similar to those found for reducing the acetylcholine response (Table I). When a mixture of equal parts of acetylcholine and histamine is used as constrictor agent, similar results are also obtained. Most of the rat preparations are insensitive to histamine. Tracheal chains of the rabbit and cat are always insensitive to histamine, even in strong concentrations (10^{-3}).

Action of Aminophylline

The relative potency of aminophylline as a bronchodilator is also shown in Table I. Since the standard adrenaline dose in any one species is about the same for antagonism of the acetylcholine and the histamine responses, it will be seen that aminophylline is a stronger antagonist to histamine contraction than it is to that produced by acetylcholine.

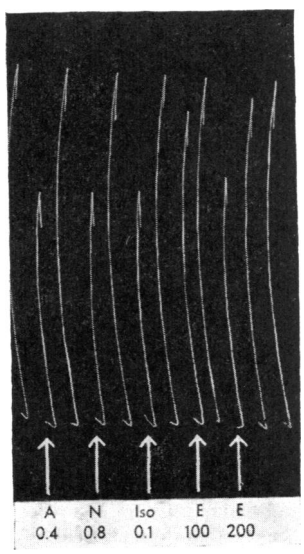

FIG. 1.—Dog tracheal preparation in 15 ml. bath. Inhibition of acetylcholine contractions (0.1 μg.) by adrenaline (A, 0.4 μg.), noradrenaline (N, 0.8 μg.), isoprenaline (Iso, 0.1 μg.), and ephedrine (E, 100 and 200 μg..) Time of contact, 1 min.

DISCUSSION

We have found that the human bronchial and the guinea-pig and dog tracheal preparations are usually sensitive to histamine, while those of the cat and rabbit entirely lack this property. There is no obvious explanation for this finding; it is not related to the histamine content of the tissues, which is relatively high in all species. The preparations reacting to histamine, however, are more sensitive to the sympathomimetic amines than are those of the cat and rabbit.

Isoprenaline is the most potent of all the bronchodilators studied, a result which agrees with the findings of previous workers using perfused guinea-pig lungs (Lands, Luduena, Ananenko, and Grant, 1950) or histamine aerosols in guinea-pigs (Chen, Portman, Russell, and Ensor, 1951). Hawkins and Schild (1951) suggest that the sympathomimetic amines and aminophylline exert a direct action on human bronchial muscle, so that isoprenaline is more active than adrenaline in this effect. Isoprenaline is much less active than adrenaline in producing relaxation of the fresh rabbit ileum (McDougal and West, 1952), so that it is possible that the point of action of these two amines is not the same in different types of smooth muscle.

The activity of noradrenaline relative to that of adrenaline varies with the species used, and it is far less in the human than in the other preparations. Hawkins and Schild (1951) found that the racemic form was about twenty-three times less active than adrenaline in producing relaxation of

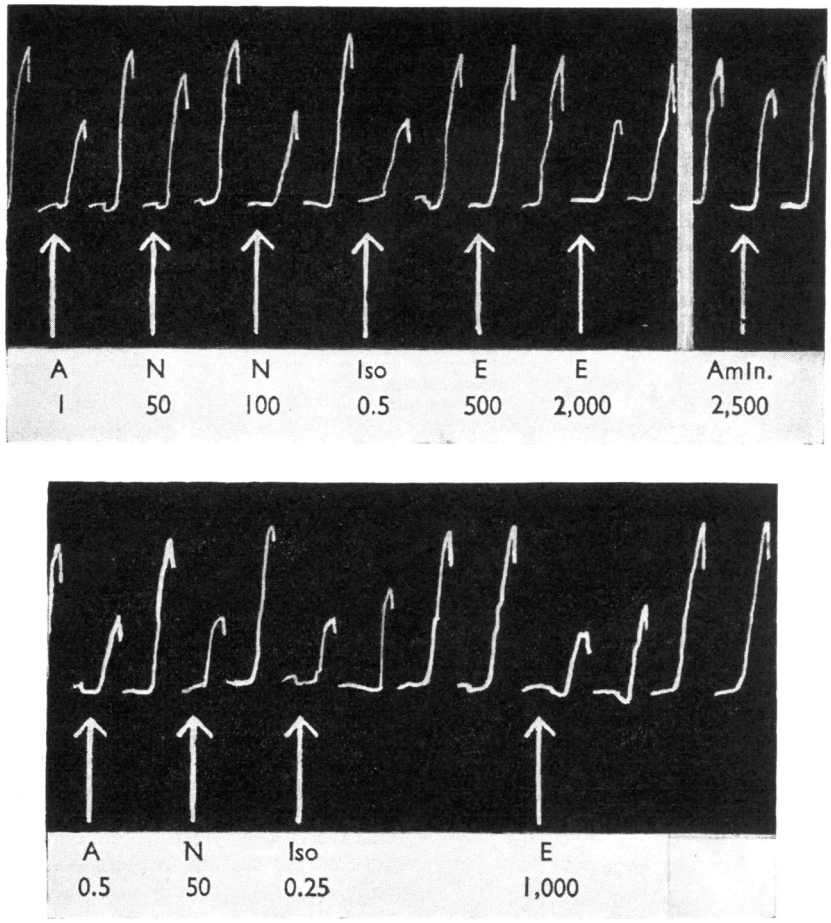

FIG. 2.—Human bronchial preparation in 15 ml. bath. Top tracing: inhibition of acetylcholine contractions (10 μg.) by adrenaline (A, 1 μg.), noradrenaline (N, 50 and 100 μg.), isoprenaline (Iso, 0.5 μg.), ephedrine (E, 500 and 2,000 μg.), and aminophylline (Amin., 2,500 μg.). Lower tracing: inhibition of histamine contractions (20 μg.) by adrenaline, noradrenaline, isoprenaline, and ephedrine. Time of contact, 1 min.

the human bronchial chain. Our results with the laevo-form show it to be at least one hundred times less active, whether the constrictor be histamine or acetylcholine. Using an *in vivo* test (antagonism of the lethal effects of a histamine aerosol in guinea-pigs), Chen, Portman, Russell, and Ensor (1951) found that *l*-noradrenaline is sixty times less active than adrenaline, whereas earlier Tainter, Pedden, and James (1934) and Luduena (1942) had reported that *dl*-noradrenaline was only seven times less active in antagonizing the histamine constriction of perfused guinea-pig lungs. The relative activity of noradrenaline therefore varies not only with the species used but also with the preparation. In man, it is much weaker than adrenaline in its action on the bronchi, whereas in the rabbit and cat it is equally potent.

In agreement with the results of previous workers, we have found that ephedrine possesses a weak bronchodilator property, especially in the human preparations. It is interesting to note, however, that like the other sympathomimetic amines it is equally potent against the effects of histamine and acetylcholine. Aminophylline, on the other hand, is more effective in preventing a histamine than an acetylcholine bronchospasm.

Large doses of ephedrine antagonize the action of adrenaline on smooth muscle, probably by combining with the adrenaline receptors (for references, see Ambache, 1951). On the other hand, effective concentrations of isoprenaline do not influence the pressor response of adrenaline in dogs (Lands, Luduena, Ananenko, and Grant, 1950). In several preparations of the bronchial muscles of

each species, therefore, we have studied the effects of continuous infusions of ephedrine and of isoprenaline on the normal antagonism of all the amines to acetylcholine and histamine. The action of aminophylline was unaffected in all preparations, whereas the bronchodilator actions of adrenaline and noradrenaline were completely prevented ; the results with acute doses of ephedrine and isoprenaline were inconsistent. This evidence suggests that the site of action of aminophylline is different from that of the sympathomimetic amines.

Summary

The relative bronchodilator actions of adrenaline, noradrenaline, isoprenaline, ephedrine, and aminophylline have been determined on the isolated tracheal rings of the guinea-pig, rat, cat, dog, and rabbit, and on the isolated human bronchial chain, after contraction of the tissues by histamine or acetylcholine. Whereas the guinea-pig, dog, and human preparations are sensitive to histamine, those of the cat and rabbit are insensitive.

Isoprenaline is the most active bronchodilator in the preparations whether the constrictor agent is acetylcholine or histamine. Although aminophylline usually is the weakest bronchodilator, it is unaffected by continuous infusions of effective concentrations of isoprenaline or ephedrine.

Noradrenaline is about one hundred times less active than adrenaline in preventing constriction of the human bronchial chain.

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