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144. Keijiro Takagi, Issei Takayanagi, and Kyo Fujie: Chemicopharmacological Studies on Antispasmodic Action. XV.[1] Non-specific Antispasmodic Action on Tracheal Muscle.

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Up to the previous report,[1] the competitive and non-competitive antiacetylcholine action were studied exclusively on the small intestines of mice. This research program was then extended to tracheal plain muscle. As the customary tracheal chain preparations[2] responded very poorly in this laboratory, connected tracheal strips, which were proved to contract more reactively than the former preparation with spasmogens such as ACh or histamine, were used. Dose-contraction curves by the agonists and the influences of competitive and non-competitive antagonists upon the contraction curves were investigated in the same way as described previously on small intestines.[3] In this report the study was concentrated on the papaverine-like non-competitive antagonistic action.

Methods

Tracheal Plain Muscle of Guinea Pig—Whole tracheal tube was excised from a male guinea pig, 500 to 900 g. in body weight, and cut open longitudinally along the anterior side of the trachea. The opened trachea was cut transversely along ring cartilages into 14 to 16 strips, which were tied up to about 5 cm. long. 7 to 8 strips are usually required and two preparations are obtained from one animal, which were suspended in a 30-cc. organ bath as in the case of small intestines.[3] The bath temperature was maintained at 38° because the preparation contracts very slowly at lower temperature. Kymographic recording of contraction by histamine is shown in Fig. 1.

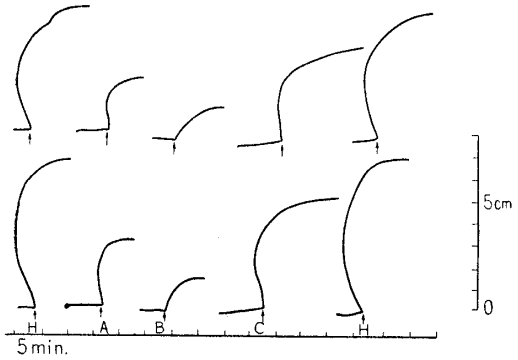

Fig. 1. Kymographic Recording of Contraction of Tracheal Strip Preparation by Histamine

H: $2\times10^{-4}M$ A: $1.7\times10^{-6}M$
B: $5.1\times10^{-6}M$ C: $1.5\times10^{-5}M$

pKa and Solubility—pKa and solubility of free bases in water were determined by titration of saturated solution with 0.04*N* HCl solution.

Results

(1) Responses of Tracheal Muscles to ACh and Histamine—Tracheal muscle has similar sensitivity both to ACh and to histamine, but the slopes of the logistic dose-contraction curves for the two agonists were different. That for ACh was about 1, and that for histamine was 1.5 (Fig. 2 and 3). The steeper curve for histamine in tracheal muscle agrees with the experiments on aortic vascular muscle by Furchgott.[4]

Now that the bath temperature was 38° and the contraction curve was obtained from the data on a single tracheal preparation, there remains no objection to the correctness of the slope for ACh and

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1) K. Takagi, *et al.*: Yakugaku Zasshi, **78**, 927 (1958).
2) J. C. Castillo, E. J. de Beer: J. Pharmacol. Exptl. Therap., **90**, 104 (1947).
3) K. Takagi, I. Takayanagi: This Bulletin, **5**, 580 (1957).
4) R. F. Furchgott: Pharmacol. Rev., **7**, 199 (1955).

histamine, while some question arises about the slope of the curve in the case of ileum, because the bath temperature was 26° and data from many preparations, which would have different sensitivity each, were averaged.

(2) **Competitive and Non-competitive Antagonism on Tracheal Muscle**—Parallel shift of a histamine dose-contraction curve and an inhibition of maximum contraction were shown by papaverine ($6\times10^{-6}M$) (Fig. 4). The situation was quite similar to the antagonism between ACh and papaverine on ileum and the competitive and non-competitive antihistaminic action can be separated, using high (10^{-3} g./cc.) and low (10^{-5} g./cc.) concentrations of histamine (Fig. 5). Then the non-competitive antihistaminic and antiacetylcholine activities of some antispasmodics were estimated on tracheal muscle and ileum according to the method previously reported[3] (Table I). On each of the 14 compounds used, non-competitive antagonistic activities to two kinds of agonists, ACh and histamine, were almost the same on tracheal muscle. The non-competitive antiacetylcholine action of the strong bases on mouse ileum was distinctly higher than the results on trachea. On the contrary, non-basic esters and weak bases such as papaverine and dihydroneuspasverine have almost the same activity, irrespective of the difference in agonists and of test organs. When the papaverine-like activity of the optical isomers of Aspaminol (1,1-diphenyl-3-piperidinobutanol hydrochloride) was compared in ileum of mice, difference in the activity was observed, while on tracheal muscles no difference was found between the optical antipodes.

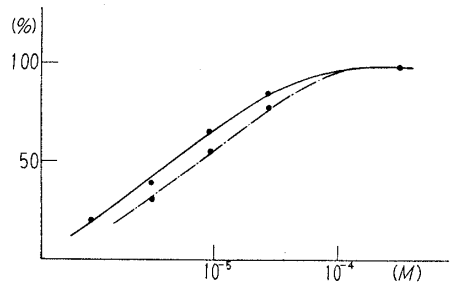

Fig. 2. Acetylcholine (ACh) Dose-Contraction Curve

———: Averaged experimental results tested on 10 tracheal preparations

- - - - - - -: Experimental results tested on single tracheal preparation

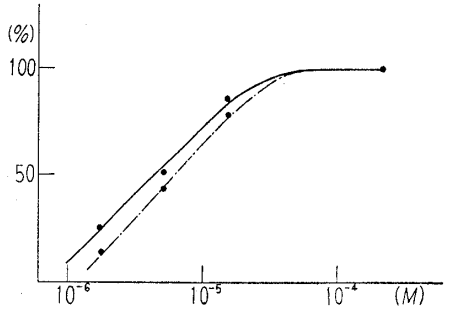

Fig. 3. Histamine Dose-Contraction Curve

———: Averaged experimental results tested on 10 tracheal preparations

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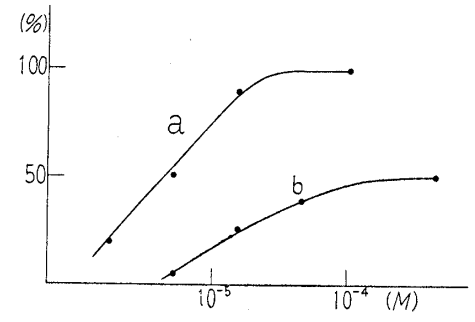

Fig. 4. Parallel Shift of Histamine Dose-Contraction Curve and Inhibition of Maximum Contraction on Tracheal Strip Preparation

a: Histamine alone

b: Histamine with papaverine ($6\times10^{-6}M$)

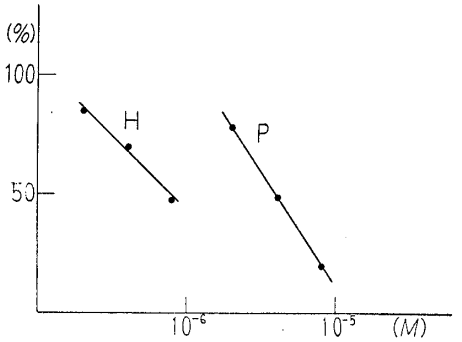

Fig. 5. Competitive and Non-competitive Antihistamine Action of Papaverine

H: Competitive antihistamine action, using low concentration (10^{-5} g./cc.) of histamine

P: Non-competitive antihistamine action, using high concentration (10^{-3} g./cc.) of histamine

Quaternary ammonium compounds have some papaverine-like activity on small intestines, for example, 2-diethylaminoethyl benzoate methiodide as 0.38% and 1-(2-dimethylaminoethyl)oxyoctane methiodide as 0.24% of papaverine, but they have no such activity on trachea up to $3\times10^{-3}M$ concentration. The competitive antihistaminic action of some antispasmodics was compared with diphenhydramine on tracheal muscle (Table III).

TABLE I. Non-competitive Antihistaminic Potency Ratio and Thermodynamic Index

	$-COOCH_2CH_2CH(CH_3)_2$	Small intestine				Trachea[c]			
		Histamine[a]		Acetylcholine[b]		Histamine		Acetylcholine	
		Index	Ratio[d]	Index	Ratio[d]	Index	Ratio[d]	Index	Ratio[d]
Group I	$(C_6H_5)_2C(OH)-$	—	—	7.1×10^2	82	7.2×10^2	80	6.7×10^2	93
	$C_6H_5CH(OH)-$	—	—	2.4×10^2	15	3.3×10^2	20	3.2×10^2	21
	$C_6H_5CH_2-$	—	—	8.3×10^2	8.5	7.1×10^2	8.8	5.9×10^2	12
	C_6H_5-	—	—	1.0×10^3	6.4	8.6×10^2	7.2	7.7×10^2	8.2
	C_3H_7-	—	—	7.7×10^2	3.0	4.0×10^2	5.6	8.3×10^2	2.7
	Dihydroneuspasverine	7.4×10^2	27	4.2×10^2	24	4.9×10^2	20	9.9×10^2	10
	Papaverine	1.5×10^2	100	1.5×10^2	100	1.5×10^2	100	1.5×10^2	100
Group II	Avacan	1.9×10^2	4.9	5.7×10^1	120	4.6×10^2	1.5	3.3×10^2	2.1
	Benactyzine	0.8×10^2	67	0.8×10^1	210	6.3×10^2	7.8	2.4×10^2	20
	d, l-Aspaminol	4.8×10^1	56	1.2×10^1	235	6.2×10^2	4.4	4.8×10^2	5.6
	l-Aspaminol	7.2×10^1	38	5.2×10^1	49	6.8×10^2	3.3	3.8×10^2	7.0
	d-Aspaminol	3.6×10^1	77	0.8×10^1	334	7.0×10^2	3.7	4.6×10^2	5.9
	2-Diethylaminoethyl benzoate	4.6×10^0	4.6	1.0×10^1	0.94	1.1×10^2	0.11	1.0×10^2	0.12
	1-(2-Dimethylaminoethyl)oxy-octane	1.2×10^1	1.9	1.6×10^1	1.5	0.95×10^2	0.24	0.79×10^2	0.29

[a] Giunea pig. [b] Mouse [c] Giunea pig. [d] Potency ratio (molar basis)

TABLE II. pKa, Saturated Concentration, and r at pH 7.8

	pKa	r	Satd. concn. (*M*)
Papaverine	5.93	0.987	6.6×10^{-5}
Dihydroneuspasverine	5.85	0.986	1.0×10^{-4}
Avacan	9.20	0.038	1.0×10^{-4}
Benactyzine	8.85	0.082	1.7×10^{-5}
d,l-Aspaminol	8.62	0.200	7.4×10^{-5}
l-Aspaminol	8.62	0.200	7.4×10^{-5}
d-Aspaminol	8.62	0.200	7.4×10^{-5}
2-Diethylaminoethyl benzoate	8.90	0.071	7.5×10^{-3}
1-(2-Dimethylaminoethyl)oxy-octane	8.87	0.079	3.4×10^{-3}
Diphenhydramine	8.98	0.074	2.0×10^{-3}

Dihydroneuspasverine: 1-Piperonyl-3-methyl-6,7-methylenedioxy-3,4-dihydroisoquinoline
Avacan: Isoamyl 2-(2-dimethylaminoethylamino)-2-phenylacetate
Benactyzine: 2-Diethylaminoethyl benzilate
Aspaminol: 1,1-Diphenyl-3-piperidinobutanol
Diphenhydramine: 2-Dimethylaminoethyl benzhydryl ether

TABLE III. Competitive Antihistaminic Potency Ratio on Trachea and Thermodynamic Index

	Potency ratio	Index
Papaverine	3.3	4.5×10^3
Dihydroneuspasverine	0.53	1.8×10^4
d,l-Aspaminol	0.21	1.3×10^3
l-Aspaminol	0.45	6.0×10^2
d-Aspaminol	0	—
Avacan	0.18	3.5×10^3
2-Diethylaminoethyl benzoate	0.10	1.2×10^2
Diethylaminoethyl benzoate methiodide	0.12	—
1-(2-Dimethylaminoethyl)oxy-octane	0.83	1.8×10^0
Benactyzine	1.3	4.3×10^2
Diphenhydramine	100	3.0×10^{-1}

(3) **Non-competitive Antihistaminic Action and pH of Bath Fluid on Tracheal Muscle**—The solubility and pKa_1 of 2-aminoquinoline were calculated from titration curve of its saturated solution. The pKa_1 is 7.26[5] and non-competitive antihistaminic activity was estimated at pH 6.6, 7.2, and 7.8, using papaverine at pH 7.8 as standard (Table IV).

TABLE IV. Non-competitive Antihistaminic Activity of 2-Aminoquinoline (pKa=7.26) at pH 6.6, 7.2, and 7.8, using Papaverine at pH 7.8 as Standard

pH	Potency ratio*	Satd. concn. (*M*)	r	Index
7.8	1.4	4.7×10^{-3}	0.760	1.2×10^{2}
7.2	0.80	4.7×10^{-3}	0.446	1.1×10^{2}
6.6	0.35	4.7×10^{-3}	0.166	1.0×10^{2}

* Papaverine=100

Thermodynamic Activity and Discussion

From the above results non-competitive antispasmodics were divided into two groups: Group I consists of non-basic compounds, such as neutral esters, and weak bases, which exist as non-ionized molecules in physiological solution, and Group II comprises strong bases such as tertiary amino compounds, which were proved to be ionized as much as 90% of the total amount in physiological solution.

Index of thermodynamic activity, $I=r/P\times S_0$,[1] was calculated each on trachea and ileum from the potency ratio, P, and the ratio r and the solubility S_0 of the base, and shown in Table I. The r of bases was obtained from their pKa and pH of the medium (Table II).

On tracheal muscle, no significant difference of thermodynamic activity is found between group I and II, when the index is derived from the neutral molecules of the basic compounds. On the contrary, the index of group II is smaller than that of group I on small intestines of mice. The latter is, however, on the same magnitude with that on trachea. These facts suggest that only some physicochemical property of neutral molecules would be concerned in non-competitive inhibition on tracheal muscle, and that the compounds in group I would exert their action on the same property both in trachea and in ileum. Parkes[6] recognized such non-specific property of papaverine, which was reasonably named "musculotropic" by him. It was found that all the compounds in group I, including papaverine, possess such a property and it should be called nonspecific inhibitory action on smooth muscles.

The compounds of group II have far higher non-competitive antiacetylcholine action on ileum as compared with the potency expected from the contents of a free base, resulting in the lower thermodynamic index than that of group I. Some quaternary ammonium salts have non-competitive inhibitory action on ileum. These phenomena can only be elucidated by the assumption that ionized molecules would participate in non-competitive inhibitory action here.[1] The difference of the activity between the optical antipodes of Aspaminol suggests some specific character of the activity on ileum. Parkes found the high antibarium activity of thiobenzilic esters,[6] especially diethylaminoethyl thiobenzilate, on guinea pig ileum, while their inhibitory action on other plain muscle preparations such as trachea, uterus, and coronary vessel was far weaker than the potency expected from the results on gut. His interpretation is that strong antibarium activity on guinea pig ileum depends on specific inhibitory action on ganglionic cells, which are located in intestinal wall and are reported to be stimulated by barium ion,[7] and that on other plain muscle preparations ganglion plays comparatively unimportant

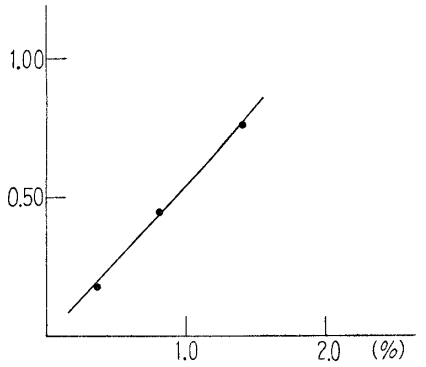

Fig. 6. Relationship between Non-competitive Antihistaminic Activity and the Free Base (r) at Different pH

Ordinate: Proportion (r) of the free base
Abscissa: Non-competitive antihistaminic potency ratio

Papaverine=100%

5) A. Albert, *et al*: J. Chem. Soc., **1948**, 2240.
6) M. W. Parkes: Brit. J. Pharmacol., **10**, 95 (1955).
7) W. Feldberg: J. Physiol., **113**, 483 (1951).

role for barium contraction. Because it is probable that affinity to ganglionic receptors is enhanced by the introduction of cationic head, results of the present experiments would confirm and further develop the conclusion made by Parkes. The findings that quaternary ammonium compounds have no non-competitive inhibitory action and that no difference in the activity was found between the optical antipodes in the case of tracheal muscle, support the opinion that even the compounds of group II exert their non-competitive activity only through a physicochemical property of the free bases there.

The compound of intermediate basicity such as 2-aminoquinoline ($pKa_1=7.26$) is partially ionized at the physiological pH and the ratio of ionization varies according to the change of pH, within which the normal responsibility of the tissue to histamine is preserved.

If the tracheal muscle is inhibited non-specifically only by the free base of 2-aminoquinoline, the potency must be reduced in proportion to lowering of pH of the bath fluid. The results in Fig. 6 show that non-competitive antihistaminic activity relates linearly with the fraction of the free base at the different pH.

Summary

(1) Tracheal strip preparation was proved to respond very sensitively to acetylcholine (ACh) and histamine. It is more suitable to test drug action on tracheal smooth muscle than tracheal chain.

(2) From the present experimental results and on applying Ferguson's rule, the papaverine-like antispasmodic action, which is tested ordinarily against barium contraction of ileum, must be divided into two categories; (a) the nonspecific inhibitory action, which is exerted by some physicochemical property of non-ionized molecules and can be tested on tracheal muscle, and (b) the specific unsurmountable inhibitory action, which is exhibited by ionized molecules of strong bases and can be tested on ileum.

(3) Neutral esters such as isoamyl esters and weak bases such as papaverine exist as non-ionized molecules in physiological pH, and they have only nonspecific inhibitory action on any smooth muscles.

Strong bases such as tertiary amines are ionized as much as 90% of the total amount in physiological solution. They exert specific but unsurmountable inhibitory action on ileum by their cation and exhibit non-specific action on tracheal muscle through their neutral base.

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