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154. Masayasu Kimura*: Chemicopharmacological Studies on Antispasmodic Action. XVII.[1] Effect of Hydrogen Ion Concentration upon Atropine- and Papaverine-like Actions.

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In the preceding paper[1] it was shown that hydrogen ion has the effect of competitive inhibition on acetylcholine (ACh). It would be most interesting, as the next step, to study the relationship between hydrogen ion and atropine (Atr) which was reported in the earlier paper[2] of this series to inhibit ACh competitively. The present investigation was initiated with the expectation that the combined effect of H^+ and atropine would perhaps be additive.

Theoretical interpretation of the additive effect is shown by the following equation (1):

$$[A]=K_A\left(1+\frac{[B]}{K_B}+\frac{[C]}{K_C}\right)\frac{y}{y'-y} \qquad (1)$$

where [A] is concentration of agonist, [B] and [C] the concentration of competitive antagonists, K_A, K_B, and K_C are dissociation constants of each molecule and its receptor, y the response in the presense of [A], [B], and [C] together, and y' the maximum response.

The increase in $[H^+]$ makes the dose-inhibition curve of atropine shift to lower concentration, in accordance with the following equation (2).

$$\frac{[B]}{K_B}+\frac{[C]}{K_C}=\left(\frac{[A]}{K_A}+1\right)\frac{y''-y}{y} \qquad (2\text{-}1)$$

$$y''=\frac{[A]/K_A}{[A]/K_A+1}y' \qquad (2\text{-}2)$$

It may be assumed that the H^+ must produce very little, if any, effect on non-competitive antagonists like papaverine (Pap). The results showing the difference of reaction between competitive and non-competitive antagonists against H^+ are also given in this paper.

The experimental method was the same as that in the preceding paper.[1]

Results

I. Synergistic Inhibitory Effect of Hydrogen Ion and Atropine on Dose-Response Curve of ACh —In order to find the effect on dose-response curve of ACh, data on $1.16\times10^{-8}M$ of Atr and at pH

Table I. Data of Synergistic Inhibition of Atropine and Hydrogen Ion

	ACh (M)	Atr (M)	pH	Response (%)
a	2.75×10^{-4}		7.4	100.0
	1.1×10^{-7}		7.4	30.4
	5.5×10^{-7}		7.4	80.5
b	4.4×10^{-7}		5.6	23.3
	2.2×10^{-6}		5.6	73.2
c	1.1×10^{-6}	1.16×10^{-8}	7.4	21.2
	5.5×10^{-6}	1.16×10^{-8}	7.4	70.1
d	4.4×10^{-6}	1.16×10^{-8}	5.6	21.8
	2.2×10^{-5}	1.16×10^{-8}	5.6	77.1

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1) Part XVI. M. Kimura: This Bulletin, **7**, 837(1959).
2) K. Takagi, M. Kimura: *Ibid.*, **5**, 440(1957).

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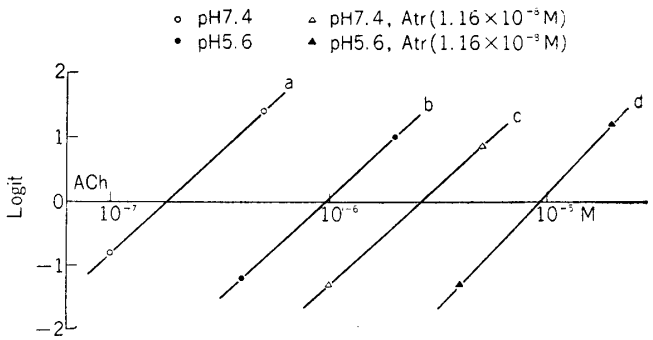

Fig. 1. Synergistic Inhibitory Effect of Hydrogen Ion and Atropine on Dose-Response Curve of ACh

5.6 are given, obtained by using 10 mice. The results of the experiment are shown in Table I and Fig. 1.

From Fig. 1, it will be seen that the dose-response curve of ACh shifted to the right due to the presence of Atr and H^+. The logistic lines from these results are almost parallel and even the response inhibited by both agents recovered to the maximum contraction with the higher doses of ACh, so that this effect may be considered as a competitive inhibition in agreement with equation (1). The effect is represented as the ratio shown in Table II.

Table II. Ratio of ACh necessary for Contraction to One-half the Maximum Response, with or without Atropine on Different pH

Atr (M) \ pH	7.4	5.6	Ratio
0	1.0	5.56	5.56
1.16×10^{-8}	15.1	51.1	3.38
Ratio	15.1	9.19	

From equation (1), if H^+ and Atr combine on the same site of the receptor, ACh necessary to contract to one-half the maximum response might be about 20 times larger with Atr at pH 5.6 than ACh at pH 7.4 without Atr. When the two agents act on different sites, the combined effect must be $15.1 \times 5.56 \fallingdotseq 84$. The experimental value was 51.1, which is intermediate between the two values.

II. Synergistic Effect of both Atr and 1,1-Diphenyl-3-piperidino-1-butanol Hydrochloride (ASP) —As a control test, experiments were conducted using 7 mice, in order to determine the typical additive effect between Atr and Asp in concentration which exerts atropine-like action. The results are shown in Tables III and IV, and in Fig. 2.

Table III. Data of Synergetic Inhibition of Atropine and Asp

	ACh (M)	Atr (M)	Asp (M)	Response (%)
a	2.75×10^{-4}			100.0
	1.1×10^{-7}			36.1
	4.4×10^{-7}			72.7
b	5.5×10^{-7}	5.8×10^{-9}		30.3
	2.2×10^{-6}	5.8×10^{-9}		76.2
c	5.5×10^{-7}		8.4×10^{-8}	28.1
	2.2×10^{-6}		8.4×10^{-8}	69.2
d	1.4×10^{-6}	5.8×10^{-9}	8.4×10^{-8}	35.6
	5.5×10^{-6}	5.8×10^{-9}	8.4×10^{-8}	78.9
	5.5×10^{-4}	5.8×10^{-9}	8.4×10^{-8}	96.1

Table IV. Combined Effect of Atr and Asp on Dose-Response of ACh

Art (M) \ Asp (M)	0	8.4×10^{-8}	Ratio
0	1.0	6.3	6.3
5.8×10^{-9}	5.4	12.3	2.3
Ratio	5.4	2.0	

If these synergistic effects obey equation (1), the theoretical value of the additive inhibition is about 11.7. Consequently, the relationship between Atr and Asp showed an additive effect, as was expected.

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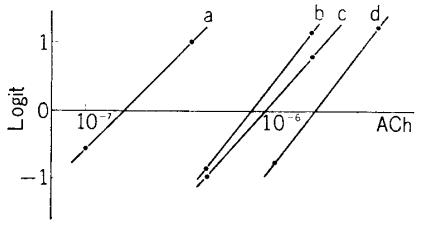

Fig. 2. Combined Effect of Atropine and 1,1-Diphenyl-3-piperidino-1-butanol on Dose-Response Curves of ACh

a) Ach
b) Ach and Atr
c) Ach and Asp
d) Ach, Atr, and Asp

III. Effect of pH on Dose-Inhibition Curve of Atr—Using 7 mice, the dose-inhibition curve of Atr was observed at pH 5.6 and 7.4 to the constant concentration of $2.2\times10^{-6}M$ of ACh. The results are shown in Table V and Fig. 3.

Table V. Effect of pH on Atropine

pH 7.4		pH 5.6	
Atr (M)	Response (%)	Atr (M)	Response (%)
5.76×10^{-7}	83.63	1.15×10^{-9}	77.63
2.88×10^{-6}	35.90	5.75×10^{-9}	48.43
		2.88×10^{-8}	12.07

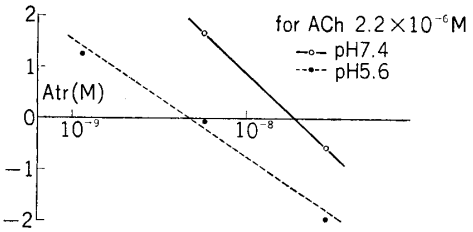

Fig. 3. Influence of pH on Atropine Inhibition Curve

From Fig. 3 it is clear that the dose-inhibition curve of Atr shifted markedly to lower dose of Atr by varying from pH 7.5 to 5.6.

IV. Effect of pH on Atropine- and Papaverine-like Action of Asp—Using 5 mice, observations were made for comparison of the effect of pH on atropine- and papaverine-like actions of Asp. The results are shown in Table IV and Fig. 4.

Table VI. Effect of pH for Atropine- and Papaverine-like Action of Asp

pH	Atr-like action		Pap-like action	
	Dose (M)	Response (%)	Dose (M)	Response (%)
7.4	1.1×10^{-7}	80.4	1.7×10^{-6}	80.9
	5.6×10^{-7}	15.9	1.0×10^{-5}	44.3
5.6	4.2×10^{-8}	72.1	1.7×10^{-6}	79.3
	2.1×10^{-7}	23.7	1.0×10^{-5}	42.3

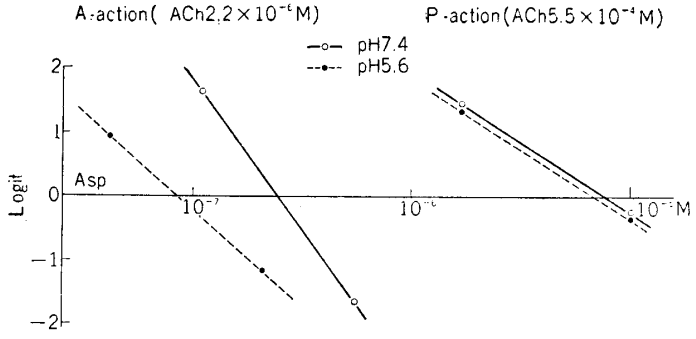

Fig. 4. Influence of pH on Atropine-like and Papavevine-like Inhibition Curves of 1,1-Diphenyl-3-piperidino-1-butanol

As will be seen from Fig. 4, the effect of pH on atropine-like action agrees with the effect on Atr, and the increase of H^+ concentration has produced very little effect, if any, on papaverine-like action.

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Discussion

Because the pKa of Atr and of Asp is 9.56 and 8.62, respectively, and as more than 95% of the agent is ionized under pH 7.4, the effect of the increased H^+ concentration upon the response of intestines is attributable to its effect on the plain muscle, especially limited to ACh-receptor within pH 5.5~7.4.[1] The inhibitory activity of Atr was augmented at a lower pH and the synergistic effect of the two agents was between the addition and potentiation (Table II). Since H^+ has only competitive antagonistic action to ACh without depression of the maximum contraction at pH 5.6, it must be additive to Atr according to equation (1). This strongly opposes the experimental results mentioned above, where H^+ was proved to possess some potentiating factor. In order to investigate the adequacy of equation (1), experiments were conducted in order to determine the combined effect of Atr and Asp. In this case the two agents have just an additive effect (Table III) and consequently they may act on the same site. While there is a considerable degree of similarity between the present author's results and those reported by Leitch,[3] some of whose conclusions were erroneous in stating that the synergistic action of H^+ and Atr was additive.

There appears to be one possible explanation to account for the curious behavior of H^+; that is to say, the affinity between Atr and the receptor is so weak that H^+ may combine freely with the Atr-receptor complex, but that the ACh-receptor complex does not do so with H^+ without its dissociation into the free receptor. Moreover, according to the "spare receptor theory" of Stephenson,[4] another explanation is possible. Even when H^+ might have some non-competitive inhibitory action, it might show an apparently surmountable antagonism to ACh by Stephenson's theory and, on the other hand, H^+ and atropine might be unsurmountable to each other, so that they show some potentiated inhibition.

Finally, between atropine- and papaverine-like actions of Asp, the former showed the same potentiation with H^+ as Atr, while the latter was hardly affected by H^+ within the range of pH 5.6~7.4. It seems possible to conclude that the specific receptor for papaverine-like action has the nature of strong acidity or that the presence of the receptor must be denied.

The author is indebted to Prof. K. Takagi of the University of Tokyo for suggesting this investigation as well as for constant guidance during the course of this work,

Summary

(1) The competitive inhibitory action of atropine to acetylcholine was augmented at a lower pH. The synergistic effect of atropine and hydrogen ion was between addition and potentiation. On the contrary, 1,1-diphenyl-3-piperidino-1-butanol was proved to have an additive effect to atropine in the range of concentration where it has only atropine-like action. A discussion on the curious behavior of H^+ on ACh receptor is presented.

(2) The atropine-like activity of 1,1-diphenyl-3-piperidino-1-butanol was subjected to the same potentiation with H^+ as atropine and the papaverine-like activity of the same compound was hardly affected by H^+ within the range of pH 5.6~7.4. It was concluded that a specific receptor for papaverine-like action has a strong acidity or that the presence of the receptor must be denied.

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3) J. L. Leitch, *et al.*: J. Pharmacol. Exptl. Therap., **120**, 408(1957).
4) R. P. Stephenson: Brit. J. Pharmacol., **11**, 379(1956).

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