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223. Keijiro Takagi, Issei Takayanagi,*1 and Chu Kun Shih*2 : A New View on the Site of Action of Pilocarpine and Arecoline in the Isolated Guinea Pig Ileum.

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The mode of action of pilocarpine, arecoline and l-ascorbic acid was studied on the isolated ileum of the guinea pig. It was given as a conclusion that pilocarpine and arecoline accelerated the acetylcholine liberation to contract the ileum and that l-ascorbic acid depressed the acetylcholine liberation from the guinea pig ileum.

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Takagi and Takayanagi[1~5] have recently proposed that with regards to the liberation of acetylcholine from the guinea pig ileum, two mechanisms can be responsible: the first is important at low frequencies of electrical stimulation and is concerned with the action of 5-hydroxytryptamine, picric acid and nicotine, and is depressed by morphine and strychnine but the nerve path way concerned with 5-hydroxytryptamine may be different from that concerned with nicotine in this mechanism,[6,7] the second is induced at high frequencies of electrical stimulation and by the action of phenyl acetate and is resistant to the inhibitory action of morphine and strychnine. Furthermore the authors have indicated that choline and its analogues accelerate the acetylcholine liberation from the cholinergic nerve of the isolated guinea pig ileum,[8] and that isoamyl acetate contracts the guinea pig ileum through the second mechanism mentioned above.[5]

In this paper the site of action of pilocarpine and arecoline was investigated on the guinea pig ileum, using the techniques of cooling the ileum and of treatment of the ileum with procaine. Furthermore the effect of l-ascorbic acid on the action of pilocarpine and arecoline and on the contraction of the electrically stimulated ileum was studied.

Methods

Guinea Pig Ileum——The experiments were made on 3 to 4 cm. strips of the male guinea pig (400 to 500 g. in body weight) ileum suspended in Tyrode solution, gassed with 95% oxygen and 5% carbondioxide. The responses of the gut were recorded on a smoked paper. The bath of 40 ml. capacity was usually maintained at 32°. In some experiments the temperature of the bath fluid was lowered to 10 to 12° for 1 to 1½ hr.[3~6] Electrical stimulation was carried out according to the previous reports.[5,9] Rectangular current pulses of 1 msec. duration and of sufficient strength were applied to the electrodes; the intraluminal electrode was made as the anode.

Frog Rectus Abdominis Muscle——The rectus abdominis muscle isolated from the female frog of 30 to 40 g. in body weight was suspended in the same organ bath containing Ringer solution at 26°, through which passed a mixture of 95% oxygen and 5% carbondioxide.

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All results were collected from at least ten experiments.

Drugs Used——Acetylcholine chloride, atropine sulfate, arecoline hydrochloride, eserine salicylate, hexamethonium bromide, histamine hydrochloride, 5-hydroxytryptamine creatinine sulfate, morphine hydrochloride, nicotine bitartrate, phenyl acetate, picric acid, pilocarpine hydrochloride, procaine hydrochloride and l-ascorbic acid. All the solutions were used after neutralized with sodium bicarbonate to pH 7.0 to 7.8.

Results

1. The site of action of pilocarpine and arecoline.

Guinea Pig Ileum

1-1. The effects of atropine, eserine and hexamethonium.

Contractions induced by arecoline and pilocarpine were competitively inhibited by atropine sulfate (10^{-8} g./ml.) and were potentiated by eserine salicylate (3×10^{-8} g./ml.) Eserine salicylate (3×10^{-7} g./ml.) contracted the guinea pig ileum in this experiment. But morphine hydrochloride (10^{-5} g./ml.) and hexamethonium bromide (10^{-5} g./ml.) were without any effect on the contractions induced by pilocarpine and aerocoline.

1-2. The effect of cooling the ileum to 10 to 12°.

After it was confirmed that on the ileum suspended in the organ bath kept at 10 to 12° for 1 to $1^1/_2$ hr., the action of acetylcholine was unaffected or potentiated and the action of nicotine, 5-hydroxytryptamine, picric acid and phenyl acetate was abolished or greatly reduced, which accelerated the acetylcholine liberation to contract the ileum, pilocarpine or arecoline was applied on the same ileum. The action of pilocarpine and arecoline was greatly reduced as shown in Fig. 1.

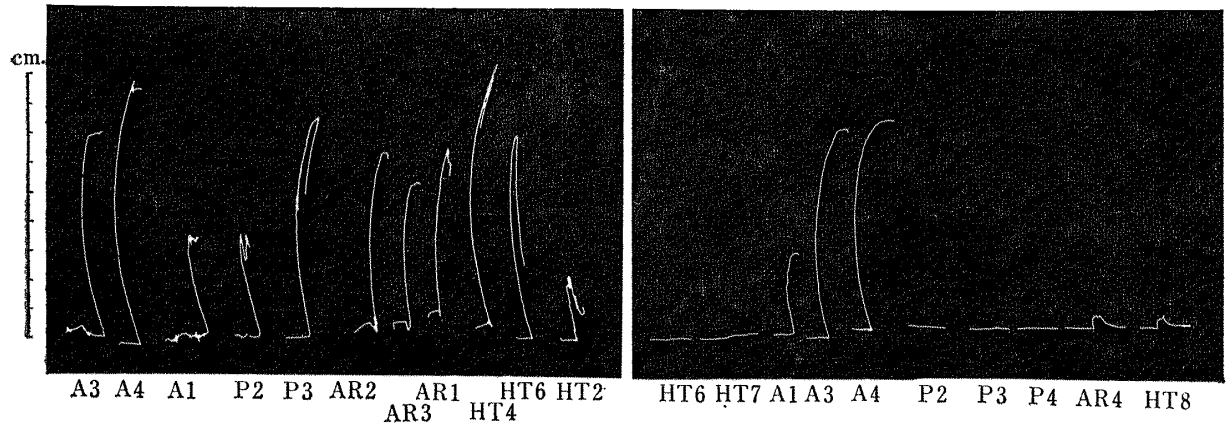

Fig. 1. The Effects of Cooling the Ileum to 10 to 12° on the Contractions induced by Pilocarpine (P), Arecoline (AR), 5-Hydroxytryptamine (HT) and Acetylcholine (A)

Meaning of marks in Fig. 1, 2 and 4 is as follows:
acetylcholine chloride (g./ml.). A1: 3×10^{-9}, A2: 6×10^{-9}, A3: 10^{-8}, A4: 3×10^{-8}.
5-hydroxytryptamine creatinine sulfate (g./ml.). HT1: 3×10^{-8}, HT2: 10^{-7}, HT3: 2×10^{-7}, HT4: 3×10^{-7}, HT5: 5×10^{-7}, HT6: 10^{-6}, HT7: 2×10^{-6}, HT8: 3×10^{-6}.
histamine hydrochloride (g./ml.). H1: 10^{-8}, H2: 2×10^{-8}, H3: 3×10^{-8}, H4: 10^{-7}.
phenyl acetate (g./ml.). PH1: 3×10^{-6}, PH2: 10^{-5}, PH3: 3×10^{-5}, PH4: 10^{-4}.
picric acid (g./ml.). PA1: 10^{-4}, PA2: 3×10^{-4}, PA3: 5×10^{-4}, PA4: 10^{-3}.
nicotine bitartrate (g./ml.). N1: 2×10^{-6}, N2: 3×10^{-6}, N3: 10^{-5}, N4: 3×10^{-5}.
pilocarpine hydrochloride (g./ml.). P1: 10^{-8}, P2: 3×10^{-8}, P3: 10^{-7}, P4: 3×10^{-7}, P5: 2×10^{-6}, P6: 3×10^{-6}.
arecoline hydrochloride (g./ml.). AR1: 10^{-9}, AR2: 3×10^{-9}, AR3: 10^{-8}, AR4: 3×10^{-8}, AR5: 6×10^{-8}.

1-3. The effect of procaine.

The contractions induced by nicotine, 5-hydroxytryptamine, picric acid, phenyl acetate, pilocarpine and arecoline were greatly reduced by a 5 min. treatment of the ileum with procaine hydrochloride (10^{-5} g./ml.) (Fig. 2). But the response to acetylcholine was little affected by procaine hydrochloride (10^{-5} g./ml.).

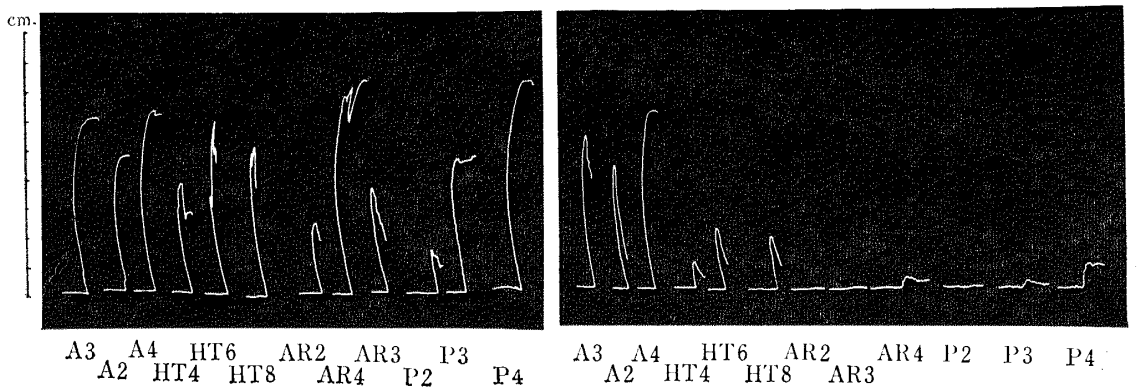

Fig. 2. The Effects of Procaine Hydrochloride on the Contractions of the Ileum induced by Pilocarpine (P), Arecoline (AR), 5-Hydroxytryptamine (HT), and Acetylcholine (A)

Left: control responses. Right: responses in the presence of procaine hydrochloride (10^{-5} g./ml.).

Frog Rectus Abdominis Muscle

1-4. The effect of pilocarpine and arecoline on the contraction by acetylcholine.

The contraction induced by acetylcholine was little affected by a 30 min. treatment of the muscle with pilocarpine hydrochloride (4×10^{-5} g./ml.) (Fig. 3) or arecoline hydrochloride (2×10^{-5} g./ml.) which did not contract the muscle. However a 20 min. treatment with eserine salicylate (2×10^{-5} g./ml.) greatly potentiated the contraction by acetylcholine (Fig. 3). This result suggests that the antiacetylcholinesterase activity of the both compounds may be very week, if any, and that at least the contraction of the guinea pig ileum produced by them was not due to their anticholinesterase activity.

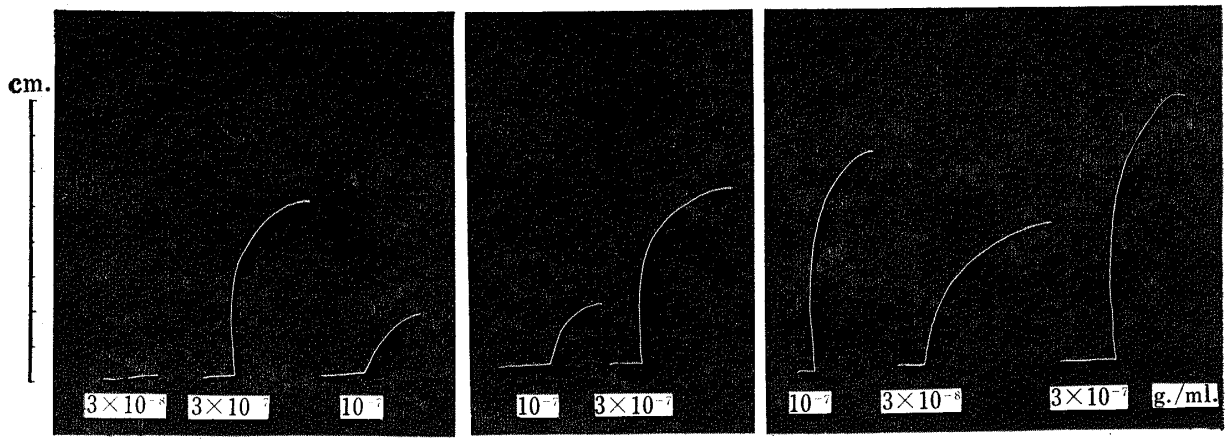

Fig. 3. The Effects of Pilocarpine and Eserine on the Contraction of the Rectus Abdominis Muscle induced by Acetylcholine

Left: control responses.
Middle: responses after a 30 min. treatment of the muscle with pilocarphine hydrochloride (4×10^{-5} g./ml.).
Right: responses after a 20 min. treatment with eserine hydrochloride (2×10^{-5} g./ml.).

It was given as a conclusion from the above results that pilocarpine and arecoline contracted the ileum through the acetylcholine liberation which was unaffected by morphine.

2. The mode of action of l-ascorbic acid tested on the guinea pig ileum.

The contractions induced by 5-hydroxytryptamine, nicotine, picric acid, pilocarpine and arecoline were greatly reduced but those by acetylcholine and histamine were not affected by 10^{-3} g./ml. of l-ascorbic acid after a 30 min. exposure of the ileum to 10^{-2}

g./ml. of l-ascorbic acid (Fig. 4). The contractions of the gut stimulated electrically was abolished completely after an exposure to l-ascorbic acid in the same way (Fig. 5).

The results suggest that l-ascorbic acid depress both the mechanisms for acetylcholine liberation, which we proposed.[3,4]

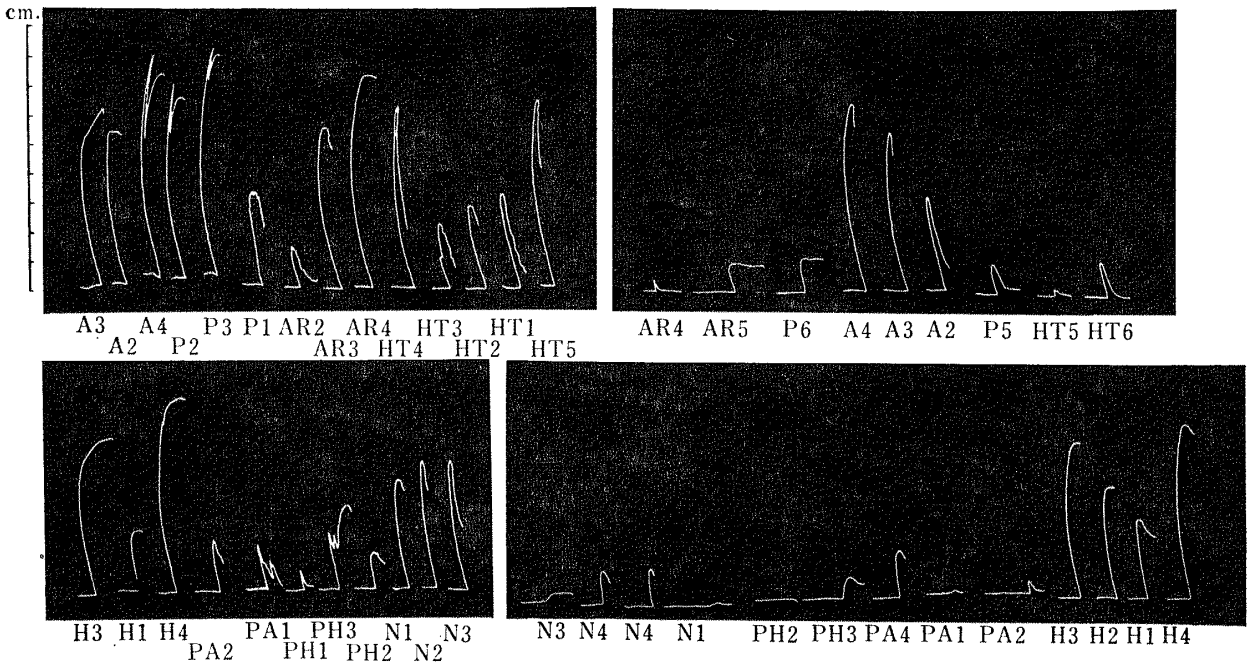

Fig. 4. The Effects of l-Ascorbic Acid on the Contractions of the Ileum induced by Pilocarpine (P), Arecoline (AR), 5-Hydroxytryptamine (HT), Picric acid (PA), Phenyl Acetate (PH), Nicotine (N), Acetylcholine (A) and Histamine (H)

Left: control responses. Right: responses after an exposure of the ileum to ascorbic acid (see text).

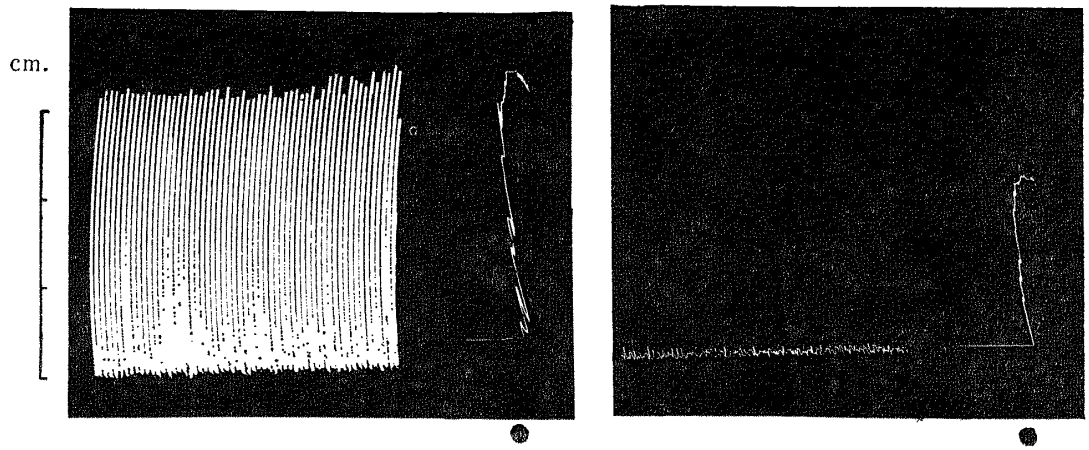

Fig. 5. The Effect of l-Ascorbic Acid on the Contractions of the Electrically Stimulated Ileum

Left: control contractions. Right: contractions after exposure of the ileum to ascorbic acid (see text).
●: acetylcholine 10^{-8} g./ml.

Discussion

When a contraction produced by an agonist is competitively inhibited by atropine but not by morphine and hexamethonium, the site of action of the agonist is usally decided to be on the acetylcholine receptor. But Takagi and Takayanagi[1~5] have recently proposed that there is also the mechanism of the acetylcholin liberation which is un-

affected by morphine (see introduction) and have indicated that isoamyl acetate contracts the ileum through the acetylcholine liberation mentioned above. Furthermore the same authors[8,10] have reported that choline, its analogues and most of the ammonium compounds may accelerate the acetylcholine liberation. In this paper we indicate that pilocarpine and arecoline which are described as typical cholinomimetics in textbooks contract the ileum through the second mechanism in acetylcholine liberation. Furthermore Takagi, Takayanagi, Taga and Nishino[11] have indicated that pilocarpine behaves as a competitive antagonist of acetylcholine, when it combines with the acetylcholine receptor. However pilocarpine was reported as a partial agonist by Takagi and Takayanagi[12] and van Rossum.[13] The results mentioned above may suggest that the site of action of cholinomimetics, especially partial agonists must be investigated more precisely, as that of the partial agonists has never been studied.

Since l-ascorbic acid inhibited only the contractions induced by the accelerators of acetylcholine liberation, such as nicotine, 5-hydroxytryptamine, picric acid, phenyl acetate and so on, it mignt nonspecifically depress both the mechanisms which we speculated on the acetylcholine liberation.

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