INHIBITORY ACTION OF SOME ANTIHISTAMINICS ON THE ANAPHYLACTIC CONTRACTION IN GUINEA PIG’S TAENIA COLI

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Since Dale’s report (1) on a hypothetical role of histamine in anaphylactic shock, the view that the histamine plays as an important chemical mediator in allergic reaction has been steadily supported by many investigators (2-10). Antihistaminics are now widely applied in allergy clinics to suppress the action of histamine produced endogenously by antigen-antibody reaction. The therapeutic effect of antihistaminics on allergic diseases is believed to be ascribed to the competition with histamine on histamine-receptor (11, 12) the drugs never acting on the immunological process itself (13). It is also acknowledged that allergic symptoms do not entirely disappear even after the administration of antihistaminics (14-16). On the other hand, it has been noted that several substances besides histamine were found to play roles in allergic shock and that antihistaminics had many pharmacological actions, in addition to antihistaminic action (17-23). Previously, Okamura (24) reported that anaphylactic contraction of taenia coli from guinea pig was dependent on the electric smooth muscle cell membrane potential. It has consequently been assumed that examination of the influence of spasmolytic drugs on membrane potential will not only have important implication for the elucidation of anaphylactic contraction, but also proved valuable clues for future study of anti-allergic drugs. In the present paper, antihistaminics were compared with adrenaline, papaverine and atropine for the purpose of elucidating the antihistaminics’ inhibitory action on anaphylactic contraction in guinea pig’s taenia coli using an electrophysiological method.

MATERIALS AND METHODS

Experimental animals: Guinepigs of Hartley strain, weighing 350-400 g, were maintained in the animal stock room, temperature 22-24°C and humidity 60%, and fed the standard solid diet (GC-4, Oriental Yeast Co., Ltd., Tokyo). Hartley males showing a wt gain during 4 weeks were used. Rabbits, weighing approx. 2 kg, were kept under the similar conditions.

Sensitization: A 10% egg-albumin in saline solution was used as sensitizing antigen. Rabbits were sensitized by subcutaneous injection of this antigen twice a week in a dose of 2 ml. A 0.1 ml of the antigen was given intracutaneously in the foot pad once a week. The sensitization was continued for 4 weeks. Two weeks after the final sensitization, antibody titration was measured by the ring method, and when precipitin titer had
multipled 2,048, the whole blood was drawn and used as anti-ovalbumin rabbit serum. About 4 ml/kg of this serum was intravenously injected to the above described guinea pigs, and after 24 hr they were employed as passively sensitized animals.

**Preparation of sample and recording:** Twenty-four hr after injection of the anti-ovalbumin rabbit serum, the animals were sacrificed and the taenia coli separated from the surrounding tissues by No. 80 cotton thread (25). The length of the used taenia coli ranged 20-30 mm in the sucrose-gap method, and 10-15 mm in the Magnus’ method. In the former method, the results were recorded by the apparatus of Stämpfli (26) and that of Burnstock et al. (27) as reported in the previous paper (24). The taenia coli isolated was fixed in the length in situ, and its membrane potential and tension were estimated under load of 1 g.

**Immunological reaction:** The qualitative precipitin reaction by the agar-plate method was performed by a modification of Ouchterlony’s procedure. For the agar-plate, a 2% agar solution, containing $10^{-4}$ g/ml antihistaminics was prepared.

Acetylcholine chloride (Daichi), histamine dihydrochloride (Junsei), atropine sulfate (Iwaki), diphenhydramine hydrochloride (Kowa), chlorpheniramine maleate (Sankyo), thonzylamine hydrochloride (Ono), adrenaline chloride (Sankyo), papaverine hydrochloride (Fujisawa) and egg-albumin (Wako) were dissolved at high concentrations in saline solution respectively. These solutions were kept in the ice box as standard and when used, were diluted in Locke’s solution.

**RESULTS**

**Effects of drugs on anaphylactic contraction**

Anaphylactic contraction was clearly visible as in Schulz-Dale’s reaction of a taenia strip isolated from the guinea pig sensitized passively (Fig. 1). The administration of a 1% egg-albumin provoked a smooth muscle contraction comparable to that induced by $10^{-6}$ g/ml of acetylcholine (ACh) or by $10^{-5}$ g/ml of histamine (Hist). In all the following experiments, the above mentioned concentrations of the drugs were used as the standard. Representing antihistaminics, were chlorpheniramine of alkylamine group, thonzylamine of ethylenediamine group and diphenhydramine of aminoether group. Concentrations of these drugs and adrenaline (Adr), papaverine and atropine which exerted 50% and 100% inhibition on anaphylactic contraction are given in Fig. 1. All the results summarized in Fig. 2 were obtained by provocation with the antigen for 5 min after pretreatment with the drugs previously described. Atropine failed to inhibit the contraction even at higher concentrations than $10^{-4}$ g/ml.

**Effects of drugs on histamine contraction**

Examinations were performed on the effects of Adr, papaverine, diphenhydramine, thonzylamine and chlorpheniramine (each $10^{-4}$ g/ml) upon the log-concentration response curve for Hist. The results are shown in Fig. 3. When the smooth muscle sample was pretreated with these drugs of $10^{-4}$ g/ml at 5 min before, % inhibition of the contraction provoked with Hist ($10^{-6}$ g/ml) were as illustrated in Fig. 4. Inhibiting
FIG. 1. Inhibitory action of adrenaline, papaverine, atropine, diphenhydramine, chlorpheniramine and thonzylamine on anaphylactic contraction in guinea pig's taenia coli (Magnus' method). ACh : $10^{-6}$ g/ml of acetylcholine, Hist : $10^{-8}$ g/ml of histamine, EA : egg albumin.
action on Hist contraction was clearly evidenced in the order of chlorpheniramine, thonzylamine, diphenhydramine, Adr, papaverine and atropine. When these results are compared with those in Fig. 2, it can be seen that the antagonistic action on Hist did not correlate with inhibitory action on anaphylactic contraction.
ANAPHYLAXIS AND ANTIHISTAMINICS

FIG. 4. % Inhibition of drugs computed from the results shown in Fig. 3.

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FIG. 5. Inhibitory action of adrenaline, papaverine, atropine, diphenhydramine, chlorpheniramine and thonzylamine (each $10^{-5}$ g/ml) on contraction induced by $\text{BaCl}_2$ ($5 \times 10^{-4}$ g/ml) in guinea pig' taenia coli.

Fig. 5. Inhibitory action of adrenaline, papaverine, atropine, diphenhydramine, chlorpheniramine and thonzylamine (each $10^{-5}$ g/ml) on contraction induced by $\text{BaCl}_2$ ($5 \times 10^{-4}$ g/ml) in guinea pig' taenia coli.
Fig. 6. Spike activity and tension recorded from guinea pig's taenia coli by the sucrose-gap method. a, Spontaneous activity; b, Inhibitory action of adrenaline, papaverine, atropine and antihistaminics on electrophysiological activity (lower trace) and tension (upper trace). For description see text.
Effects upon the contraction produced by $\text{Ba}^{++}$

The effects of Adr, papaverine, atropine, diphenhydramine, chlorpheniramine and thonzylamine on the contraction produced with $\text{BaCl}_2$ $5 \times 10^{-4}$ g/ml were examined in the concentration $10^{-5}$ g/ml by the Magnus' method (Fig. 5). $\text{BaCl}_2$-solution at the concentration mentioned above has an effect comparable to that of Hist $10^{-6}$ g/ml and ACh $10^{-4}$ g/ml. In all the cases, $\text{BaCl}_2$ was applied 5 min after pretreatment with the drugs. Atropine was entirely ineffective on the contraction produced with $\text{BaCl}_2$, whereas all the antihistamincs exerted more or less remarkable inhibitory effects.

Effects upon membrane potential and tension

The effects on membrane potential of each of the drugs which inhibited the contraction by 50$\%$ and 100$\%$ were examined by the sucrose-gap method. The results are shown in Fig. 6. Fig. 6a represented spontaneous spike activity (lower) and sion (upper). Adrenaline abolished spike activity and lowered tension at 5 sec after application in the concentration which inhibited the contraction by 50$\%$. The resting potential tended to increase slowly after the abolishment of spike, and at 5 min, spontaneous excitation occurred. Adrenaline in the concentration which completely inhibited the anaphylactic contraction, produced decrease in spike frequency and spike size, abolition of spike, hyperpolarization and drop in tension (Fig. 6a). Decrease in spike size paralleled with hyperpolarization, and caused the loss of spike activity. As late as 5 min after the administration, the spontaneous spike remained invisible, the hyperpolarization being maintained at a certain level. The same was observed with papaverine, but atropine at $10^{-4}$ g/ml did not exert any effect on the spike frequency, spike size, resting potential and tension.

On the other hand, diphenhydramine, chlorpheniramine and thonzylamine at con-
centrations of 50% inhibition elicited decrease in spike frequency, spike size and tension. Diphenhydramine, however, produced higher hyperpolarization than the other two, but conversely a more mild inhibition of spike frequency. Thonzylamine was generally evidenced to have the most remarkable action on the inhibition of spike activity (Fig. 6b).

Effects on antigen-antibody combination

Influence of the drugs above described on antigen-antibody combination were examined by means of the Ouchterlony's agar-plate method (Fig. 7). In agar, containing Adr, papaverine, atropine, diphenhydramine, chlorpheniramine, and thonzylamine to $10^{-4}$ g/ml, precipitation between egg-albumin and anti-egg albumin rabbit serum was invariably demonstrated by two thin streaks, which were not different from those produced when the agar contained 0.9% NaCl.

![Fig. 8. Hypothetic schemes for inhibitory action modes of antihistaminics on anaphylactic contraction. For description see text.](image)

DISCUSSION

Since the report on the importance of the role of Hist in allergic reaction, antihistaminics have extensively been used in experimental as well as clinical works without the allergic reaction itself being elucidated. The use is based on a simple notion that antihistaminics at high concentration will antagonize, in allergic reaction, to other substances regarded as chemical mediator, despite the fact that plurality of the mediators is now drawing attention. That intervene by Hist in allergic reaction has been confirmed by many workers (1–10). There, however, is very weak ground to assume that Hist is the only mediator (14, 16, 24, 28, 29). Concentration of the Hist, which is released into plasma during anaphylactic shock, the shock symptoms can not be provoked even in most sensitive guinea pigs (30). Therefore it appears true that antihistaminics exert
competitive antagonism to all body reactions in which Hist has been proved to intervene, however it is also true that antihistaminics are not so effective concerning allergic reactions.

Thus Hist cannot be said to be the only mediator in anaphylaxis. Nevertheless antihistaminics inhibited anaphylactic contraction in the guinea pig’s taenia coli as demonstrated in the present experiments. Adr, however, exerted an inhibitory effect at lower concentrations than antihistaminics. A similar result was obtained with the tracheal muscle (16). Moreover, inhibitory effects of antihistaminics on anaphylactic contraction did not correlate with their antihistaminic actions. This indicates that the inhibitory effect of the antihistaminics may include non-specific action besides competitive antagonism to Hist.

On the other hand, it is known that the relaxing action of Adr on smooth muscle is derived from abolition of spike and hyperpolarization (25, 31-33), and that the latter results from abolition of Ca-spike, that is, generator potential, and increase in bound-Ca, which are both produced by inhibition of Ca-influx by Adr (34, 35). Relaxing action of papaverine is said to be due to a drop in effective concentration of Ca in Ca-store (34). Furthermore, it is reported that in the relaxing actions of Adr and papaverine, the abolition of spike activity is observed predominantly, and that hyperpolarization enhances inhibition of mechanical activity (36). In the present experiment, Adr and papaverine abolished spike activity, produced hyperpolarization, and reduced tension. Also the antihistaminics demonstrated similar effects. Out of the three antihistaminics, thonzylamine was strongest in the inhibition of spike activity, and this corresponded to its inhibitory action on anaphylactic contraction. Furthermore the antihistaminics inhibited the contraction produced by BaCl₂, while they were ineffective on antigen-antibody reaction. There are also reports that catecholamines inhibit histamine-release induced by antigen administration (37-39), and it may therefore be considered that the inhibition by Adr of anaphylactic contraction is due to the suppression of Hist release. However, the fact that the contraction of the taenia coli produced by Ba⁺⁺ was inhibited by Adr indicates that this inhibition can be ascribed to the stabilizing action on plasma membrane. The same can be said for antihistaminics. All these considerations lead the authors to the assumption that spike potential or resting potential may play some important role in the inhibition of anaphylactic contraction in the guinea pig’s taenia coli. Anaphylactic contraction cannot be considered essentially different from other contractions produced by any kind of smooth muscle-contracting agent. They all appear identical in their genetic mechanism characterized by antigen-antibody reaction. To date, despite extensive use of antihistaminics in allergic reaction, nothing is clear concerning the action mechanism except competitive antagonism to Hist. It appears that the underlying mechanism is the excitation inhibition of cytoplasm membrane by suppression of ion permeation which is stimulated by antigen-antibody reaction. In other words, stabilizing action of the antihistaminics on plasma membrane is the essential nature of its inhibitory effect on anaphylactic contraction. This also explains why Adr which has stronger inhibitory action than antihistaminics on membrane potential exerts a stronger inhibitory
effect on anaphylactic contraction.

Fig. 8 illustrates the mechanism underlying the inhibition of anaphylactic contraction by antihistaminics, which was inferred from the present experiments. It has hitherto been assumed that antihistaminics competitively block the reaction of the Hist receptor on the smooth muscle cell with Hist which is released from the mast cell as the result of antigen-antibody reaction. Contrarily, the authors' view is that antihistaminics, combining with the Hist receptor, depress spike activity or resting potential, that is, plasma membrane excitability, and thus prevent antigen-antibody reaction on the plasma membrane or stimulation to muscle tissue from the product of antigen-antibody reaction. Antigen-antibody reaction on smooth muscle cell cannot be denied, since the receptor for cytophylic antibodies (40) which is found to have strong cell affinity consists of lipoproteins (41) or phospholipoproteins (42).

Glucocorticoids which are used as an antiallergic agent are said to inhibit an allergic reaction by blocking Hist release from the mast cell (43) and histamine's synthesizing and binding ability (44) without affecting antigen-antibody combination (45). Elucidation of the action mechanism of these drugs awaits future studies.

SUMMARY

With the object of investigating antiallergic action of drugs, antihistaminics were compared with adrenaline, papaverine and atropine by using the sucrose-gap method and Magnus' method, and thus was sought the role of antihistaminics in the inhibition of anaphylactic contraction in the guinea pig's taenia coli.

1. Adrenaline and thonzylamine exerted strong inhibitory action on anaphylactic contraction, whereas atropine was ineffective even in concentrations higher than $10^{-1}$ g/ml. There was no correlation between their inhibitory effects on the contraction and antihistaminic actions.

2. At concentrations which produced approx. 50% inhibition of anaphylactic contraction, all the used drugs except atropine elicited decrease in spike frequency and spike size, and moreover abolition of spike and hyperpolarization.

3. At concentrations inhibiting anaphylactic contraction, none of the used drugs affected antigen-antibody combination as examined by the Ouchterlony's agar-plate method. Diphenhydramine, chlorpheniramine and thonzylamine inhibited the contraction of the taenia coli induced by BaCl₂.

4. From these results it was supposed that the essential mechanism of inhibition by antihistaminics of anaphylactic contraction is the depression of excitation of cytoplasm membrane by blocking ion permeation, and prevention, through combination with the histamine-receptor, of antigen-antibody reaction on muscular cytoplasm membrane or stimulation of muscle tissue by its product.
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