EFFECT OF HISTAMINE ON CALCIUM MOVEMENT IN GUINEA PIG TAENIA COLI

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Calcium ions are known to play an important role in a contraction of smooth muscle. However, as reviewed by Lüllmann (1), no correlation has been found between tissue Ca content or Ca fluxes and the contractile response of the smooth muscle to various stimulants.

In guinea pig taenia coli, it was shown that cellular Ca fraction of relatively tightly bound form increase during contraction induced by barium or high concentration of potassium and decreased during the abolition of tension development by various inhibiting factors, and suggested that the shift of Ca into the relatively tightly bound fraction within the cell might have a concern with the tension development (2-5).

In this paper, it was attempted to make clear an effect of histamine on Ca exchange of smooth muscle in absence or presence of antihistamine, and the above suggestion based on the nonspecific smooth muscle stimulants was confirmed in the experiments using the specific smooth muscle stimulant.

Some of this work has already been briefly reported (6).

METHODS

Strips of taenia coli were isolated from white male guinea pigs, and immersed in the Tyrode solution oxygenated with 95% O₂ and 5% CO₂ at 37°C. The Tyrode solution had the following composition (mM); NaCl 136.8, KCl 2.7, CaCl₂ 2.5, MgCl₂ 1.0, NaH₂PO₄ 0.4, NaHCO₃ 11.9 and glucose 5.5. Isotonic-40 mM K (high-K) solution was made by replacing 40 mM NaCl by equimolar KCl.

Tension: The contractile responses were recorded isometrically by a strain-gauge transducer (Nihon Kohden).

Tissue Ca: Muscles equilibrated for 40 minutes in normal Tyrode solution were treated in a medium containing histamine at a concentration of 5.4×10^{-6} M or high-K solution. In case of antihistamine-treatment, 3.3×10^{-8} M tripeleennamine was applied to the muscles at the 15th minutes before the application of histamine or high-K solution. A certain period after the application of histamine or high-K solution, the strips were re-

moved from the bath. Then the strips were drawn across a sheet of filter paper to remove adhering solution, weighed, ashed in a furnace at 550°C and tissue Ca content was determined by an atomic absorption spectrophotometer (Perkin-Elmer Model 303).

⁴⁵Ca uptake: After pretreatment, muscles were soaked in a solution with tracer amount of ⁴⁵Ca and histamine or high-K solution. In some experiments, ⁴⁵Ca was added 15 or 30 minutes after the addition of histamine. Strips were treated in the same manner as tissue Ca determination and radioactivity was counted by a gas flow counter (Aloka Window Type Model PS-13).

⁴⁵Ca efflux: Muscles were treated for 2 hours in 5 ml radioactive Tyrode solution containing ⁴⁵Ca (20 μ Ci/ml). After loading the muscles were rinsed successively in a series of test tubes with nonradioactive Tyrode solution at one minute interval. At the end of the experiment, the radioactivities in the effluent and the muscle were counted. The rate of ⁴⁵Ca efflux was calculated from the radioactivity in each test tube and was plotted as a fraction of the initial rate. The amount of ⁴⁵Ca remaining in the muscle was also plotted.

Tightly bound fraction: As the intracellular Ca in taenia coli could not be divided into fractions from the ⁴⁵Ca efflux curve (3, 7), the Ca fraction which did not exchange within 4 minutes has been tentatively named as the "tightly bound fraction (TBF)". TBF was estimated from ⁴⁵Ca uptake (4 minutes wash) experiment. At the end of 15 or 30 minutes incubation, the muscle was washed three times during 4 minutes period with Tyrode solution of the same chemical composition as the incubation medium.

Extracellular space: Extracellular space was measured using ¹⁴C-sorbitol (4).

RESULTS

Tension changes: Fig. 1 depicts the typical tension changes seen after application of Tyrode solution containing 5.4×10^{-6} M histamine or high-K solution.

Hexamethonium at a concentration of 3.7×10^{-6} M, 3.1×10^{-7} M tetrodotoxin, 2.8×10^{-6} M atropine or 2.9×10^{-6} M scopolamine inhibited neither the action of histamine nor high-K solution. However, when the muscles were pretreated with 3.3×10^{-6} M tri-

FIG. 1. Tension change curves of taenia coli treated with 5.4×10^{-6} M histamine or high-K (40 mM, isotonic) solution.

pelennamine, the antihistamine prevented the effect of histamine without changing the effect of high-K solution. These results are consistent with the data by Akubue (8) suggesting that the action of histamine on the taenia coli of guinea pig resulted from the stimulation of specific receptors sited on the smooth muscle.

The next series of experiments were made to determine the external Ca dependence of the response of taenia coli to histamine. The muscles were kept in low Ca medium (1.25 or 0.25 mM Ca) for 20 minutes and then histamine was added. Histamine-induced tension declined according to the decrease of Ca concentration in the medium. In Ca depleted medium, no response was observed in the presence of histamine and restoration of Ca was followed by an immediate contraction of the muscle.

Tissue Ca: Ca content of the muscle in normal Tyrode solution was 4.8 ± 0.2 mEq/Kg wet tissue (8) and the value did not change significantly throughout the period of observation. In the presence of histamine, tripeleennamine or both histamine and tripeleennamine, Ca content did not differ significantly from control value (Fig. 2).

In high-K solution, tissue Ca content increased gradually to 5.6 ± 0.1 mEq/Kg wet tissue (11) during 30 minutes (Fig. 2), which was in agreement with the previous data (4, 5). The tripeleennamine treatment did not modify the increase in tissue Ca content of the muscle treated with high-K solution.

FIG. 2. Changes in tissue Ca and ^{45}Ca uptake of taenia coli in the presence of 5.4×10^{-6} M histamine (left) and high-K (40 mM, isotonic) solution (right). Histamine or high-K solution was added at time 0. Each point in graph represents the mean of 6 to 20 experiments. Standard errors of means are also noted.

Tissue Ca ($\square$), ^{45}Ca uptake ($\circ$) in Tyrode solution without (filled symbols) and with histamine or high-K solution (open symbols).

^{45}Ca uptake: ^{45}Ca uptake in the muscles are shown in Fig. 2. The figure represents that the cellular Ca mostly exchanges with extracellular ^{45}Ca within 15 minutes. ^{45}Ca uptake of the muscle did not differ from control values either when ^{45}Ca was added simultaneously with histamine or 15 minutes later than the addition of histamine. When ^{45}Ca was added 30 minutes after the addition of histamine, the slight depress in ^{45}Ca uptake was observed (Fig. 2).

The ^{45}Ca uptake did not change from the value obtained in the normal Tyrode solu-

FIG. 3. Changes in tissue Ca and ^{45}Ca uptake in the presence of 3.3×10^{-6} M tripeleennamine (left) or 5.4×10^{-6} M histamine and tripeleennamine (right).

Tissue Ca (□), ^{45}Ca uptake (○) in Tyrode solution without (filled symbols) and with drugs (open symbols).

FIG. 4. Effect of histamine on the efflux of ^{45}Ca from taenia coli.

Upper curve: Amount of radioactivity in the muscle (left ordinate).

Lower curve: Rate of ^{45}Ca efflux (right ordinate).

5.4×10^{-6} M histamine was added between two arrows.

FIG. 5. Changes in tissue Ca and TBF in the presence of histamine or histamine and tripelennamine.

Conditions	Tissue Ca	TBF
Control	■	▲
Histamine	□	△
Histamine and tripelennamine	◆	△

FIG. 6. Changes in tissue Ca and TBF in the presence of high-K solution or high-K solution with tripelennamine.

Conditions	Tissue Ca	TBF
Control	■	▲
High-K solution	□	△
High-K solution with tripelennamine	◆	△

tion in the presence or absence of histamine under tripelennamine treatment (Fig. 3).

^{45}Ca efflux: In the presence of histamine, no significant change was observed on the curves showing the rate of ^{45}Ca efflux and the amount of radioactivity remaining in the muscle (Fig. 4).

Tightly bound fraction (TBF): Fig. 5 and 6 show the changes in TBF of the muscle. The size of TBF after 30 minutes incubation was 1.0 ± 0.1 mEq/Kg wet tissue (24) in normal Tyrode solution and increased to 2.7 ± 0.2 mEq/kg (6) in histamine-added solution and to 2.4 ± 0.1 mEq/Kg (7) in high-K solution.

When the muscle was pretreated with tripelennamine, histamine did not develop tension and the size of TBF stayed at the control level (Fig. 5). However, the tripelennamine pretreatment did not alter the increase in the size of TBF of the muscle treated with high-K solution (Fig. 6).

Extracellular space: The extracellular space measured with ^{14}C -sorbitol was $36.3 \pm 0.9\%$ (7) in normal Tyrode solution. When muscles were incubated with histamine or histamine and tripelennamine, the extracellular space was $34.8 \pm 1.7\%$ (7) and $34.1 \pm 1.2\%$ (8), respectively. The extracellular space was scarcely influenced by histamine or histamine and tripelennamine.

These results were schematically summarized in Fig. 7.

FIG. 7. Schematic arrangement of Ca spaces in taenia coli under various conditions. Size of TBF is superimposed upon the column of intracellular space.

DISCUSSION

Histamine induced a tonic contraction and increased the size of TBF but did not affect tissue Ca content or Ca fluxes in taenia coli. The slight depress in ^{45}Ca uptake observed 30 minutes after the addition of histamine may reflect the increase in the size of TBF, i.e., the increase in slowly exchanging Ca fraction in the tissue. Tripelennamine treatment of the muscle abolished both histamine-induced contraction and the increase in the size of TBF. From the above data, it is suggested that loosely bound cellular Ca moves towards tightly bound Ca fraction during histamine induced contraction of taenia coli.

In the case of high-K induced tonic contraction, it has been reported that both Ca influx and tissue Ca content increased and the Ca ion taken up in the cell moved towards TBF (2, 3). On the other hand, during Ba induced tonic response, although tissue Ca content remained unchanged and both fluxes decreased, cellular Ca moved towards TBF within the cell (3). Comparing these and the present data, the changes in tissue Ca contents and Ca fluxes are rather conflicting during the tonic contractions induced by these smooth muscle stimulants. Similar observations have been reviewed by Lüllmann (1) on the smooth muscles in the presence of various stimulants and relaxants. However, the presence of the correlation between tension development and the size of TBF has already been suggested (3-5) and the present data also supports this.

The examination of time courses of the increase in tension development and the size of TBF shows that the tension reaches almost maximal level soon after the addition of histamine and maintained at a steady level, but the size of TBF increased gradually during the period. The similar time courses have been observed in the cases of high-K and Ba induced tension developments (3). Further, it was observed that although the tonic

tension development in high-K solution declined within one minute when the medium was replaced with normal Tyrode solution, the increased size of TBF decreased gradually to the control level within 15 minutes (unpublished data), suggesting that there is no direct correlation between tension and the size of TBF.

From these data, it may be proposed that the increased intracellular Ca ions resulted from the increase in Ca influx by high-K solution or from the release of bound Ca by histamine or Ba interact with contractile protein to induce contraction, from which the Ca ion is taken up continuously by TBF to maintain contraction.

The tension development in taenia coli by histamine was accompanied by a proportional increase in the rate of oxygen consumption, both of which were abolished by anti-histamine, the relation between oxygen consumption and Ca movement in the presence of histamine will be reported and discussed in detail in the following paper (9).

SUMMARY

The effect of 5.4×10^{-6} M histamine on Ca exchange was investigated in guinea pig taenia coli.

1. In the presence of histamine, Ca content, ^{45}Ca uptake and ^{45}Ca efflux did not differ considerably from control value, but the size of cellular Ca fraction which did not exchange within 4 minutes (tightly bound fraction, TBF) increased.

2. When the muscle was pretreated with 3.3×10^{-6} M tripeleennamine, histamine did not induce contraction and the size of TBF was maintained at the control value. The pretreatment with tripeleennamine did not alter the increments in tension, tissue Ca content and TBF by high-K solution.

3. It may be suggested that histamine releases loosely bound cellular Ca and, at least, a part of the Ca ions interact with contractile protein to induce contraction, from which Ca is taken up continuously by TBF to maintain contraction.

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