EFFECT OF CHOLINERGIC DRUGS AND BARIUM ON OXYGEN CONSUMPTION IN GUINEA PIG TAENIA COLI

Yukio SAITO and Yutaka SAKAI
Medical Laboratory for Pharmacology, Central Research Laboratories,
Sankyo Co., Ltd., Shinagawa-ku, Tokyo

Norimoto URAKAWA
Department of Veterinary Pharmacology, Faculty of Agriculture,
University of Tokyo, Bunkyo-ku, Tokyo, Japan

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It has been reported that taenia coli isolated from guinea pig showed tension development accompanied by an increase in oxygen consumption when treated with a stimulant such as acetylcholine, eserine (1), histamine (1, 2) or high concentration of potassium in medium (3). An increase in oxygen consumption during histamine or high-K induced contraction is probably linked to increased Ca ions in the cytoplasm of the muscle cell (2, 4).

The present experiments were undertaken to investigate effects of carbachol, pilocarpine, atropine and barium on oxygen consumption, muscle tension and electrical activity in taenia coli.

METHODS

Strips of taenia coli were isolated from male guinea pig, Hartley strain, weighing approx. 500 g and suspended immediately in a perfusion chamber which was equipped with an apparatus for simultaneous recording of medium partial oxygen pressure (Po2) and changes in muscle tension and electrical activity. This apparatus has been described previously (2, 3). Tyrode solution was of the following composition (mM); NaCl, 136.8; KCl, 5.4; CaCl2, 2.5; MgCl2, 1.0; NaH2PO4, 0.4; NaHCO3, 11.9 and glucose, 5.5. For high-K solution 40 mM NaCl was substituted by 40 mM KCl in the composition. Carbachol chloride (Aldrich Chemical Co., Inc., U.S.A.), pilocarpine hydrochloride (Tokyo Chemical Industry Co., Ltd., Tokyo), atropine sulfate (Tokyo Chemical Industry Co., Ltd., Tokyo) and barium chloride (Koso Chemical Co., Ltd., Tokyo) were dissolved in Tyrode solution. Ethyleneglycol bis (β-aminoethylether)-N,N'-tetraacetic acid (EGTA) at a concentration of 0.1 mM was added to Ca-depleted solution to eliminate residual Ca ions. All solutions for perfusion were kept at 37°C and equilibrated at pH 7.2 with 95% O2 and 5% CO2.

RESULTS

After incubation for 30 min, a tension of 0.2 g was applied to the muscle strip. Twenty min later, the muscle was pretreated with 40 mM K for 8 min. Muscles bathed in the normal Tyrode solution for 45 min reached a steady state level in mechanical, electrical and re-
FIG. 1. Effects of carbachol and atropine on medium Po2, electrical activity and muscle tension of taenia coli.

The upper, middle and lower curves show electrical activity (µV), Po2 level (mmHg) and muscle tension (g), respectively.

The dot line indicates the Po2 level without the muscle in chamber.

Time in min represents the time after the muscle was suspened in the chamber.

The wet wt of taenia coli was 34.9 mg.

Between A and B, carbachol (1 x 10^-6 M) was added.

Between C and D, K concentration was elevated isotonically to 45.4 mM.

From E on, medium was supplemented with atropine (5 x 10^-6 M).

spiratory activities while some muscles showed spontaneous contraction and exhibited rhythmic fluctuations in electrical and respiratory factors. An addition of 40 mM K to the medium induced a tension development of the muscle to approx. 10 g, a disappearance of electrical activity and a significantly lowered Po2 level in perfusate to approx. 550 mmHg within 15 min (Fig. 1).

Effect of cholinergic drugs: When normal Tyrode solution in the perfusion system was
replaced by $1 \times 10^{-6}$ M carbachol-containing one, the muscle showed a tension reaching to about 12 g and then declined gradually. During the contraction, electrical activity increased in frequency and amplitude, while the $P_{O_2}$ level decreased to about 510 mmHg in 15 min (Fig. 1). Fifteen min later the carbachol-containing solution was replaced by the normal Tyrode solution, and the three activities returned to the original levels after about 15 min. When atropine was added to Tyrode solution at a concentration of $5 \times 10^{-6}$ M, the three activities in the muscle were scarcely affected. Addition of $1 \times 10^{-6}$ M carbachol to the

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**Fig. 2.** Effects of pilocarpine and atropine on medium $P_{O_2}$, electrical activity and muscle tension of taenia coli.

Refer to the note in Fig. 1.

The wet wt of the muscle was 35.8 mg.

Between A and B, pilocarpine ($4 \times 10^{-7}$ M) was added.

Between C and D, K concentration was elevated isotonically to 45.4 mM.

From E on, medium was supplemented with atropine ($5 \times 10^{-6}$ M).
solution containing atropine did not change the three activities. On the other hand, when high-K solution containing atropine was perfused, the muscle developed tension to about 11.5 g while its electrical activity ceased and Po2 level decreased to approx. 570 mmHg within 15 min. The results were almost similar to those obtained with the high-K Tyrode solution without atropine.

Pilocarpine at a concentration of $4 \times 10^{-6}$ M showed similar effects on the three activities to those shown by carbachol, which was also completely antagonized by an addition of $5 \times 10^{-3}$ M atropine (Fig. 2).

**Effect of barium (Ba):** On addition of barium to the normal medium at a concentration of 1.5 mM the muscle developed tension to about 12 g. The sustained contraction was different from that after high-K in that it was accompanied by rapid superimposed rhythmic contractions. Electrical activity increased and Po2 level decreased rapidly to 450 mmHg.

![Fig. 3. Effects of barium (Ba) on medium Po2, electrical activity and muscle tension of taenia coli.](image)

Refer to the note in Fig. 1.

The wet wt of the muscle was 33.9 mg.

Between A and B, K concentration was elevated isotonically to 45.4 mM.
Between C and D, Ba (1.5 mM) was added.
Between E and F, Ba (0.5 mM) was added.
during contraction, as shown in Fig. 3. After washing out Ba, the frequency and height of the rhythmic contractions gradually decreased and Po$_2$ level gradually returned to the original level in 90 min. The electrical and mechanical activities and oxygen consumption changed almost in parallel. A lower concentration of Ba, 0.5 mM, induced similar changes in contraction and electrical activity but to a less degree than those by 1.5 mM Ba. The sustained contraction which had a tension of about 3 g soon changed into a rhythmic one of high amplitude, and electrical activity also changed into a rhythmic one. The Ba induced reproducible changes in the muscle.

In another series of experiments (Fig. 4), the normal Tyrode solution was exchanged for the Ca-depleted one containing 0.1 mM EGTA (Ca(O)-solution). The first application of 2.5 mM Ba to the muscle was performed at the same time as the Ca depletion. The second

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**Fig. 4. Effects of Ca removal from medium on Ba-induced changes in medium Po$_2$, electrical activity and muscle tension of taenia coli.**

Refer to the note in Fig. 1.

The wet wt of the muscle was 37.5 mg.

a: Perfusion fluid was normal Tyrode solution.

b: Perfusion fluid was Ca(O)-solution.

Between A and B, Ba (2.5 mM) was added.
application induced less mechanical and electrical activities, but a greater change in Po₂ level than those in the normal medium with Ba. The third and fourth applications induced more or less a similar degree of tension attaining about 2 g within 15 min. The muscle exhibited only a few spike discharges, delayed onset of contraction, and decreased in Po₂ level during these applications of Ba. In the third application the muscle showed a lesser decrease in Po₂ level than the others. When Ca was re-added to the medium, an application of 2.5 mM Ba enhanced its effects on the three activities. From these data, it was observed that the Ca removal from medium decreased both the tension development and the extra respiration induced by the application of 2.5 mM Ba.

The muscle was suspended in Ca(O)-solution flowing through the bath at the beginning of experiment. Sixty min later, Ca(O)-solution was changed to Ca(O)-high K solution,

![Graph](image1)

**Fig. 5.** Effects of external Ca and Ba on medium Po₂ and muscle tension following the application of 40 mM K in Ca-depleted Tyrode solution in taenia coli.

Refer to the note in Fig. 1.
The wet wt of the muscle was 34.8 mg.
a: Perfusion fluid was the Ca(O)-high K solution.
Between A and B, Ca (2.5 mM) was added.
Between C and D, Ba (2.5 mM) was added.
then the latter solution was perfused for 30 min. When 2.5 mM Ca was added to Ca(O)-high K medium, the muscle increased tension to about 12 g and PO_2 level decreased to 460 mmHg (Fig. 5). The first application of 2.5 mM Ba for 15 min to the muscle kept in Ca(O)-high K solution induced muscle tension of 4.8 g, and a slightly less decrease in PO_2 level than that in Ca(2.5 mM)-high K medium. The second and third applications induced muscle tension of 3.8 g and PO_2 level of about 510 mmHg, which were less than the changes by the first application. When Ca(2.5)-high K solution was exchanged with Ca(O)-high K 30 min after washing out Ba in the third application, the muscle developed tension to 11.5 g and the PO_2 level decreased to approx. 490 mmHg within 15 min. These results indicate that Ba added to Ca(O)-high K medium at a concentration of 2.5 mM caused a slightly less increase in oxygen consumption than that in the Ca(2.5 mM)-high K medium, but developed much less muscle tension in the latter medium. Thus, Ba-substitution for Ca appears to disassociate the increase in oxygen consumption from muscle tension change in smooth muscle treated with high K.

DISCUSSION

As the apparatus employed in the present experiment was capable of recording the spike discharge but not the DC membrane potential (2), this data showed only that carbachol at a concentration of 1 x 10^-6 M, pilocarpine at a concentration of 4 x 10^-6 M and Ba in a concentration range from 0.5 mM to 2.5 mM increase frequency and amplitude in spike discharge, and that high-K solution induced a transient increase followed by a disappearance of electrical activity as a result of depolarization. On the other hand, acetylcholine in a concentration range from 10^-7 g/ml to 10^-5 g/ml was reported to cause a slow depolarization and increase in spike frequency of the smooth muscle in taenia coli (5-7). From all these data, effects of the cholinergic drugs on electrical activity in taenia coli were observed to be almost similar.

In Bübring's report (8) atropine in concentrations from 10^-6 to 3 x 10^-6 g/ml caused no significant change in spontaneous activity, except for an initial relaxation and slowing of the spike discharge, while the action of acetylcholine was completely prevented. In this experiment, atropine inhibited the effect of carbachol and pilocarpine on the electrical and mechanical activities of the muscle, though it did not induce an initial relaxation and slowing of the spike discharge.

Suzuki et al. recorded electrical activity of taenia coli by the intracellular technique and reported that Ba at a concentration of 1.4 mM increased the spike frequency, which was followed by prolongation of the duration in spike potential of the muscle in taenia coli. When the concentration of Ba was increased to 2.2 mM, the membrane potential fell promptly and the spike discharge became faster and irregular, ceasing after 30 sec exposure to Ba (9). On the contrary, present data indicates that Ba at a concentration of 2.5 mM does not show a cease of spike discharges, but rather an increase in amplitude and frequency. The inconsistency between these data may be due to different recording techniques on electrical activity of the muscle and also to high concentrations of Ba employed.
in this experiment.

On the other hand, Ba added to the Ca-free solution, at a concentration of 2.5 mM, did not increase frequency and amplitude of spike discharge but developed tension to 2 g. Bülbüning and Tomita reported that the membrane of the muscle soaked in Ca-free solution was depolarized and spikes and tension were very small or abolished. When Ba at a concentration of 1 mM was added to the Ca-free solution, the muscle revealed spontaneous activity. Ba concentration was increased to 2.5 mM, after which the spike amplitude and duration further increased forming a long-lasting plateau or a maintained state of depolarization (10). The transient spike discharge followed by infrequent small spikes during Ba treatment observed in the present experiment are in agreement with these observations using the double sucrose-gap method (10).

Carbachol and pilocarpine increased tension of the muscle which was accompanied by an increase in oxygen consumption. Atropine completely inhibited both changes. On the other hand, acetylcholine and eserine were reported to show similar results on oxygen consumption in smooth muscle (1). In taenia coli, cholinergic drugs induce a tension development accompanied by an increase in oxygen consumption.

In earlier works (2, 4), an application of histamine or an elevation of K concentration in the medium caused an increase in tension development accompanied by an increase in oxygen consumption of the muscle. In the study on Ca movement in smooth muscle, histamine shifted Ca ions from a loosely bound fraction to a more tightly bound one (11), and high-K solution increased Ca ions which enter the depolarized muscle through the membrane during the tonic contraction (12, 13). In both cases, the increased Ca ions in the cell are assumed to accelerate metabolic process(es), resulting in the increase of oxygen consumption (2, 4). Cholinergic drugs, such as carbachol, pilocarpine and acetylcholine increased the exchange of Ca ion in early phase of contraction, and released cellular Ca and subsequently accumulated it into a more tightly bound fraction in the late phase. In the presence of atropine, cholinergic drugs did not induce any tension development nor change in Ca movement in the cell (14). These and present data on the cholinergic drugs seem to confirm the proposal that increased Ca ions in cytoplasm of the muscle cell stimulate metabolic process(es) thereby increasing oxygen consumption (4).

Ba had a similar effect on Ca movement of the smooth muscle during contraction (13), and the increased Ca ions in the cell appear to participate in tension development and increase in respiration.

On the other hand, an application of 2.5 mM Ba developed small tension, but increased oxygen consumption of the muscle soaked in the Ca(O) or Ca(O)-high K medium. It was reported that the Ba ion revealed much less activity in contraction of the glycinerated muscle of taenia coli than that shown by Ca ion (15), and that Ba ion also had an extremely small affinity to troponin prepared from chicken gizzard (16). Accordingly, it is unlikely that Ba ions directly contribute to the tension development in taenia coli suspended in Ca(O) or Ca(O)-high K medium, but sequestered Ca ions may be released by Ba, resulting in the tension development suggested in an earlier paper (17).
On metabolism, Ba ion was reported to substitute for Ca ion in the activation of phosphorylase b kinase (18), however, there are no reports that Ba ion substitutes for Ca ion in the activation of mitochondrial respiration. It is possible that the extra respiration of the muscle treated with 2.5 mM Ba in Ca(O) or Ca(O)-high K medium is caused by Ca ions released by Ba from the sequestered site and/or Ba ion entering cytoplasm through the cell membrane, however, present data are in sufficient to support this assumption.

SUMMARY

Effects of cholinergic drugs and barium on mechanical, electrical and respiratory activities were investigated in the smooth muscle of guinea pig taenia coli.

1. Carbachol at a concentration of $1 \times 10^{-6}$ M and pilocarpine at a concentration of $4 \times 10^{-6}$ M induced tension development, an increase in electrical activity and a decrease in PO, level. These three activities changed almost in parallel.

The effects of the cholinergic drugs on the three activities were completely inhibited by atropine at a concentration of $1 \times 10^{-6}$ M.

These results seem to support the proposal that the increased Ca ions in cytoplasm of smooth muscle cell participate in muscle contraction and also stimulate metabolic process(es) resulting in an increased oxygen consumption.

2. Barium in a concentration from 0.5 mM to 2.5 mM showed almost similar effects on the three activities as was shown by the cholinergic drugs. After an application of 2.5 mM Ba, a poor tension was developed with a great consumption of oxygen of the muscle soaked in the Ca(O) or Ca(O)-high K medium.

These results are insufficient to discuss whether or not the extra respiration of the muscle treated with 2.5 mM Ba in Ca(O) or Ca(O)-high K medium is caused by Ca ion released by Ba from the sequestered site and/or Ba ion entering cytoplasm through the cell membrane.

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