EFFECT OF CHOLINERGIC DRUGS ON CALCIUM MOVEMENT IN GUINEA PIG TAENIA COLI

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Abstract - The effect of cholinergic drugs on Ca movement was investigated in guinea pig taenia coli. In the presence of carbachol (1 x 10^{-6} M), Ca content and extracellular space did not differ from control value, ^45Ca uptake and efflux increased in the "early phase" and the size of cellular Ca fraction which did not exchange within 4 min (tightly bound fraction, TBF) increased in the "late phase" in the contraction. When the muscle was pretreated with atropine (1 x 10^{-6} M), carbachol did not induce the contraction and ^45Ca uptake and efflux in the "early phase" and the size of TBF in the "late phase" was maintained at the control value. The effect of pilocarpine (5 x 10^{-6} M) and acetylcholine (1 x 10^{-5} M) on Ca movement was the same with carbachol. In summary, cholinergic drugs increase Ca exchange and continuously accumulate the cytoplasmic Ca ions into the slow exchanging fraction. Changes in Ca movement by various stimulants were classified into three types, 1, increase in Ca exchange, 2, increase in Ca net influx and subsequent accumulation and 3, release of cellular Ca and subsequent accumulation.

It is a current view that a rise in free intracellular Ca activates contraction and a fall in cytoplasmic Ca activity leads to relaxation in both striated and smooth muscles. It has already been suggested that the activator Ca is provided from an influx of extracellular Ca or from a release of Ca at a cellular site during a contraction induced by 40 mM K (1, 2), Ba (2) and histamine (3) in the smooth muscle of guinea pig taenia coli, and that the Ca ion acting on contractile protein is actively and continuously accumulated in a slowly exchanging Ca fraction and the continuous flow of Ca ion through the contractile element is related to the continuation of tension development (4).

On the other hand, the possibility that the cholinergic drugs alter the permeability of the cell membrane to monovalent-ion has been considered (5), however the relationship between Ca movement and tension development has not been established. The present experiment was done to clarify an effect of cholinergic drugs, i.e., carbachol, pilocarpine and acetylcholine, on Ca movement in guinea pig taenia coli.

MATERIALS AND METHODS

Strips of taenia coli were isolated from male Hartley strain guinea pigs weighing about 500 g, and immersed in Tyrode solution bubbled with 95% O_2 and 5% CO_2 at 37°C. The solution contained (mM): NaCl 136.8, KCl 2.7, CaCl_2 2.5, MgCl_2 1.0, NaH_2PO_4 0.4, NaHCO_3 11.9 and glucose 5.5.

Tension: Contractile responses were recorded isometrically by a force-displacement

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**Tissue Ca:** After equilibration for 40 min in normal Tyrode solution, muscle strips were treated in a medium containing a cholinergic drug, i.e., $1 \times 10^{-6}$ M carbachol, $5 \times 10^{-6}$ M pilocarpine or $1 \times 10^{-5}$ M acetylcholine. In case of the treatment with a cholinergic blocking agent, $1 \times 10^{-6}$ M atropine was applied to muscles 15 min before the application of a cholinergic drug. At a certain period after the application of cholinergic drugs, strips were removed from the bath. Adhering solution was removed by drawing the strips across a sheet of filter paper. Then the strips were weighed, ashed in a furnace at 550°C and tissue Ca content was determined by an atomic absorption spectrophotometer (Perkin-Elmer Model 303).

**$^{45}$Ca uptake:** After the equilibration, strips were soaked in a solution with $^{45}$Ca and a cholinergic drug. This experiment was performed to examine Ca uptake at the initiation of the contraction by cholinergic drugs, and this period was named "early phase". In some experiments, $^{45}$Ca was added 15 min after addition of the cholinergic drug to examine Ca uptake in the "late phase". After the $^{45}$Ca incubation, strips removed from the bath were treated in the same manner as the determination of tissue Ca. Radioactivity was counted by a gas flow counter (Aloka Window Type Model PS-13).

**$^{45}$Ca efflux:** Strips were treated for 2 hr in 20 ml radioactive solution containing $^{45}$Ca ($20 \mu$Ci/ml). The strips were then washed successively in a series of test tubes containing nonradioactive Tyrode solution at an interval of 2 min. At the end of the wash period radioactivities in the effluent and the strips were counted. The rate of $^{45}$Ca efflux was calculated from the radioactivity in each test tube and was plotted as a fraction of the initial rate. The amount of $^{45}$Ca remaining in the strips was also plotted.

**Tightly bound fraction (TBF):** It has been shown that Ca ion acting on contractile protein is accumulated actively in a slowly exchanging Ca fraction during the tonic contraction in taenia coli, which exchanges in an exponential process with a half time of about 7 min (4). Increase in the size of the slowly exchanging Ca fraction is pronounced at 4 to 6 min of the Ca desaturation curve (4). In the present experiment, the Ca fraction the dose of which does not exchange within 4 min, and corresponds to the value at 4 min of the Ca desaturation curve, was measured. The Ca fraction which has been named as the "tightly bound fraction (TBF)" was estimated as follows; at the end of 15 or 30 min incubation in a medium containing a cholinergic drug and $^{45}$Ca, the strips were washed 3 times during a 4 min period with Tyrode solution of the same chemical composition as the incubation medium and the amount of $^{45}$Ca remaining in the muscle was determined. The time schedule of the TBF experiment was planned to estimate the change of the Ca fraction in the "late phase".

**Extracellular space (ECS):** Muscle strips were incubated in a solution with or without a cholinergic drug for 30 min and $^{14}$C-sorbitol was added during the last 15 min. After the incubation period, muscle strips were treated with solubilizer (Soluene TM-100, Packard Co.) and radioactivity was counted using a liquid scintillation spectrometer (Packard type 2311) (6).
RESULTS

Tension changes: Fig. 1 shows the typical tension changes seen after the application of $1 \times 10^{-6}$ M carbachol, $5 \times 10^{-6}$ M pilocarpine or $1 \times 10^{-5}$ M acetylcholine. The action of these cholinergic drugs was not affected by $3.1 \times 10^{-6}$ M tetrodotoxin. However, cholinergic drugs did not induce the contraction in the presence of $1 \times 10^{-6}$ M atropine.

Tissue Ca: Ca content of the muscle in normal Tyrode solution was $4.8 \pm 0.2$ mEq/kg wet wt. of the tissue (8). The cholinergic drugs scarcely affected the tissue Ca level through the period of observation in the presence or absence of atropine (Figs. 2-A and B, 4 and 5).

$^{45}$Ca uptake: $^{45}$Ca uptake of the muscle increased when $^{45}$Ca was added simultaneously with carbachol, pilocarpine or acetylcholine. However, the pretreatment with atropine inhibited the increase in $^{45}$Ca uptake of the muscles soaked in Tyrode solution containing the cholinergic drugs (Figs. 2-B, 4 and 5). When $^{45}$Ca was added 15 min after the addition of carbachol or pilocarpine, $^{45}$Ca uptake of the muscles did not differ from control level (Figs. 2-A and 4). It was observed that in the "early phase" of the cholinergic effects, there was an increase in $^{45}$Ca uptake but a change in $^{45}$Ca uptake in the "late phase" was not detectable.

Fig. 1. Tension change curves of taenia coli treated with $1 \times 10^{-6}$ M carbachol, $5 \times 10^{-6}$ M pilocarpine and $1 \times 10^{-5}$ M acetylcholine.
FIG. 2. Changes in tissue Ca and \(^{45}\text{Ca}\) uptake of taenia coli in the presence of \(1 \times 10^{-6} \text{M}\) carbachol (A) or carbachol and \(1 \times 10^{-6} \text{M}\) atropine (B).

Each point in the graph represents the mean of 7 to 20 experiments. Standard error of the mean is also noted.

\(^{45}\text{Ca}\) efflux: When the muscles were treated with carbachol for 4 min, both efflux curves shown in Fig. 3 exhibited an increase in \(^{45}\text{Ca}\) efflux. Thus, carbachol increased the Ca efflux in the “early phase”. In the presence of atropine, carbachol did not increase \(^{45}\text{Ca}\) efflux.

Tightly bound fraction: The size of TBF after 30 min incubation was \(1.0 \pm 0.1 \text{ mEq/kg wet tissue} \) (24) in normal Tyrode solution. By the addition of carbachol or pilocarpine, the size of TBF after 15 min incubation did not change from the control value, but after 30 min incubation the size of TBF increased significantly. When muscles were pretreated with atropine, the size of TBF remained at the control level (Figs. 2-B, and 4).

In the presence of carbachol or pilocarpine, Ca content did not differ from control value and the slowly exchanging Ca fraction increased in the “late phase”; i.e. these drugs decreased Ca exchange in this phase.

Extracellular space: ECS measured with \(^{14}\text{C}-\text{sorbitol}\) was \(36.3 \pm 0.9 \% \) (7) in normal Tyrode solution. ECS was scarcely influenced by carbachol \((35.8 \pm 2.5 \% \) (8)) or pilocarpine \((35.1 \pm 1.5 \% \) (8)).
Fig. 3. Effect of carbachol on $^{44}$Ca efflux from taenia coli.
Upper curve: Amount of radioactivity in the muscle (left ordinate).
Lower curve: Rate of $^{44}$Ca efflux (right ordinate).
$1 \times 10^{-6}$ M carbachol was added between two arrows.

Fig. 4. Changes in tissue Ca, $^{44}$Ca uptake and TBF in the presence of $5 \times 10^{-6}$ M pilocarpine or pilocarpine and $1 \times 10^{-5}$ M atropine.
In summary, the cholinergic drugs increased the rate of Ca exchange during the "early phase", and rather decreased it during the "late phase" in taenia coli.

DISCUSSION

Several reports have been published concerning the effect of cholinergic drugs on Ca movement in intestinal smooth muscle. However, these results are conflicting and the cholinergic effect on Ca movement has not been established. One of the reasons for the confusion may be attributed to the fact that the cholinergic effect on Ca movement is composed of two different phases; "early" and the "late phase", as shown in the present experiment. On this basis, an attempt was made to divide these inconsistent data into the "early" and the "late" phase.

Schatzmann (7) reported that the change in a net Ca uptake could not be ascertained 20 min after the addition of 1x10^{-5} g/ml acetylcholine in guinea pig taenia coli. This time schedule corresponds to the "late phase" in the present experiment. In Ca efflux experiment (7, 8), ^{45}Ca loaded muscles were washed with a normal solution for 30 min and then with a solution containing acetylcholine and an increase of ^{45}Ca efflux was observed, which corresponds to the "early phase". Hattingberg et al. (9) showed that in taenia coli, 1x10^{-4} g/ml pilocarpine had no effect on Ca content, ^{45}Ca uptake and inulin space, which corresponds to the "late phase". In ^{45}Ca efflux experiment, muscles were loaded in ^{45}Ca medium with pilocarpine for 30 min, and then washed successively with non-radioactive medium containing pilocarpine. The results showed that the drug had no effect on the Ca desaturation curve, and suggested that the drug scarcely affected the Ca distribution in taenia coli during the "late phase". Their results were mostly confirmed by the present experiment except for a slight increase observed in the slowly exchanging Ca fraction. In the strips of guinea pig ileum, Chujyo and Holland (10) showed that 5x10^{-4} M pilocarpine increased the slow exchanging Ca fraction which did not exchange during 5 min, and there was no demonstrable effect on ECS. The data are consistent with those
of the present authors. Although the former authors reported a decreased tissue Ca and no demonstrable effect on $^{45}\text{Ca}$ efflux by pilocarpine, there is no available datum to explain the difference from ours. Banerjee and Lewis (11) noted in guinea pig ileum that $1\times10^{-4}\text{g/ml acetylcholine}$ increased $^{45}\text{Ca}$ uptake and efflux, and that $6\times10^{-6}\text{g/ml carbachol}$ increased $^{45}\text{Ca}$ uptake and did not affect on $^{45}\text{Ca}$ efflux. As there is no precise description on the time schedule of application of the drugs, it is impossible to judge whether these results correspond to the “early” or “late phase”.

There are several reports on the effect of cholinergic drugs on Ca movement in depolarized smooth muscle. Robertson (12) reported that depolarized strips of rabbit ileum still contracted by $1\times10^{-6}\text{g/ml acetylcholine}$ and the drug increased $^{45}\text{Ca}$ uptake, but the time schedule of the experiment was not described. Durbin and Jenkinson (13) also showed that $3\times10^{-7}\text{g/ml carbachol}$ developed additional tension in depolarized guinea pig taenia coli and both $^{45}\text{Ca}$ uptake and efflux increased within 10 min of addition of carbachol. Potter and Sparrow (14) found that the rate of Ca loss in Ca-free solution was augmented by acetylcholine in the depolarized circular muscle of cat small intestine. In the depolarized toad’s stomach muscle (15), no difference in the $^{45}\text{Ca}$ content was observed in muscle containing its full complement of Ca, but Ca uptake, efflux or Ca content was increased when Ca-depleted muscle was exposed to Ca 0.05 mM solution with $5\times10^{-3}\text{M acetylcholine}$. Further, when Ca-depleted muscle was exposed to Ca 0.05 mM solution for 30 min with acetylcholine, increase in the slowly exchanging Ca fraction was detected.

Analyzing the various data on both polarized and depolarized smooth muscle of various kinds, changes in Ca movement in the smooth muscle by cholinergic drugs is summarized to indicate that the drugs increased the rate of Ca exchange in the “early phase” and then increased the slowly exchanging Ca fraction in the “late phase”.

It is proposed that the cholinergic drugs increase the membrane permeability to Ca ion and increase available Ca ions in cytoplasm to initiate the muscle contraction, and that the Ca ion activating the contraction is accumulated continuously to the slowly exchanging Ca fraction, that is, a flow of Ca from membrane to the slowly exchanging Ca fraction via a contractile element contributes to maintain a contraction. The gradual increase in the size of the slowly exchanging Ca fraction results in a gradual decrease in the rate of Ca exchange.

The effect of histamine and cholinergic drugs on Ca movement (3), oxygen consumption (16, 17) and electrical activity (16, 17) are compared in Table 1. The most pronounced difference between the effects of these drugs is that histamine produced only an increase in the slowly exchanging Ca fraction during the contraction (3). Although it has been shown that the action potential of smooth muscle cell is associated with an inward movement of Ca ion (18) and both drugs increased spike frequency, an increase in Ca influx was observed only in the “early phase” of cholinergic drugs. The different effect of these drugs on the Ca movement cannot be attributed to the technical difference in each experiment. Lammel and Golenhofen (19) also reported that electrical stimulation of taenia coli did not induce any increase in $^{45}\text{Ca}$ uptake of the muscle. The data on hista-
mine suggest the possibility that the Ca spike may be induced by release of membrane bound Ca.

From the studies reported so far, the contraction of taenia coli by various stimulants was divided into two phases, phasic and tonic contraction. It is considered that the change in Ca movement in the “early” and the “late phase” corresponds to the phasic and the tonic contraction, respectively, and the changes in the movement of Ca observed in the presence of various stimulants are classified as shown in Table 2. The first type is seen in the “phasic contraction” of the cholinergic drugs and barium (2); the increase in the rate of Ca exchange without affecting tissue Ca level. The second type is a rare case seen only in the “tonic contraction” of 40 mM potassium; the increase in net Ca uptake. The third type is seen in the “phasic contraction” of 40 mM potassium (2) and also in the “tonic contraction” of histamine (3), barium (2) and cholinergic drugs; no effect or rather de-

### Table 1. Effects of histamine or cholinergic drugs on various factors in the smooth muscle of guinea pig taenia coli.

| Cholinergic drugs | Histoamine | Carbachol | Pilocarpine | Acetylcholine |
|-------------------|------------|-----------|-------------|--------------|
|                   | $5.4 \times 10^{-5}$ M | $1 \times 10^{-6}$ M | $5 \times 10^{-6}$ M | $1 \times 10^{-5}$ M |
|                   | Early     | Late     | Early     | Late     | Early     | Late     | Early     | Late     | Early     | Late     |
| Tissue Ca         | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ |
| ECS               | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ | $\rightarrow$ |
| Uptake            | $\rightarrow$ | $\downarrow$ | $\uparrow$ | $\rightarrow$ | $\uparrow$ | $\rightarrow$ | $\uparrow$ | $\rightarrow$ |
| Efflux            | $\rightarrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ |
| TBF               | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ |
| $Q_0^2$           | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ |
| Spike frequency   | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ | $\uparrow$ |

ECS: Extracellular space, TBF: Tightly bound Ca fraction, $Q_0^2$: Oxygen consumption, Early, Late: Early phase, Late phase
$\uparrow$: Increase, $\downarrow$: Decrease, $\rightarrow$: No change

For details see text. (From Saito, Sakai, Ikeda and Urakawa (16), and Saito, Sakai and Urakawa (17): Change in Ca movement by histamine from Nasu, Karaki, Ikeda and Urakawa (3)).

### Table 2. Types of change in Ca movement during contraction induced by various stimulants in taenia coli.

| Types of Ca movement | Stimulants |
|----------------------|------------|
| Increase in Ca exchange | Cholinergic drugs (P) |
|                      | Ba (P) |
| Increase in Ca net influx and subsequent accumulation | 40-K (T) |
| Release of cellular Ca and subsequent accumulation | Cholinergic drugs (T) |
|                      | Histamine (T) |
|                      | 40-K (P) |
|                      | Ba (T) |
crease in the rate of Ca exchange without changing tissue Ca level. The second and third types of the Ca movement are accompanied by the increase in the slowly exchanging Ca fraction. As the second type is to increase both Ca influx and slowly exchanging Ca fraction, this type may be intermediate between the first and the third type. It is now generally agreed that the increase in the cellular free Ca ion contributes to the tension development and all the types of the changes in Ca movement by the stimulants listed in Table 2 explain the increase in cellular Ca during the contraction of intestinal smooth muscle.

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