COMPONENTS COUPLING CHOLINERGIC EXCITATION WITH CONTRACTION OF SMOOTH MUSCLES OF GUINEA PIG TAENIA COLI

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Acetylcholine (AC) in concentrations of 10^{-9}-10^{-7} g/ml increases the smooth-muscle tone of the guinea pig taenia coli by increasing the permeability of the cell membranes to inward flows of Ca^{45} ions. In concentrations of 10^{-6} g/ml or higher AC induces the liberation of membrane calcium and, in concentrations of 10^{-5}-10^{-3} g/ml, it significantly increases the membrane permeability of the smooth-muscle cells to Na^{22} ions, causing depolarization and an increase in action potential frequency. It is postulated that the mechanism coupling the cholinergic stimulus with the final effect (muscle contraction) includes three components: increased entry of Ca^{++} into the smooth-muscle cells, liberation of membrane calcium, and a spike mechanism.

KEY WORDS: smooth muscles; acetylcholine; ionic permeability; electrical and mechanical activity; coupling components.

Recording electrical phenomena is a method used to determine the characteristics of cholinergic reception in skeletal muscles and neurons [2, 3, 9]. However, electrical activity evidently is not an adequate parameter with which to analyze the effect of cholinomimetics on smooth muscles, for the latter respond by contraction to application of agonists even in a depolarizing solution, i.e., when electrogenic mechanisms are inhibited [11].

The object of this investigation was to analyze processes coupling the cholinergic stimulus with contraction of the smooth muscles of the guinea pig taenia coli.

EXPERIMENTAL METHOD

Isolated strips of guinea pig taenia coli were immersed in Krebs's solution at 37°C with continuous oxygenation. Contractions were recorded under isotonic conditions with a load of 1 g. Acetylcholine (AC) was used in concentrations of between $1 \cdot 10^{-9}$ and $1 \cdot 10^{-3}$ g/ml. Some experiments were carried out in sodium-free sucrose solution. To study the entry of Na^+ into cells of the taenia coli the strips were placed for 0.5 min in a solution containing 5 μCi/ml Na^{22} and the agonist in the concentration for testing; in experiments with Ca^{++} the strips were incubated for 2 min in solution containing 2.5 μCi/ml Ca^{45}. To remove Na^{22} from the extracellular space, the preparations were washed for 15 sec in distilled water, treated for 10 min with a mixture of formalin and calcium chloride, and rinsed for 15 min in a jet of water. For the same purposes, in experiments with Ca^{45} the strips were treated for 3 min with Na_2EDTA ($1 \cdot 10^{-3}$ g/ml), immersed for 10 min in formalin, and then washed for 15 min in tap water. The radioactivity of weighed samples of strips mineralized in HCl was measured on the UMF-1500 M apparatus (for Ca^{45}) and the PP-8 radiometer (for Na^{22}). Electrical activity was investigated by the single sucrose-gap method. A smooth-muscle preparation measuring 0.5 × 0.5 × 15 mm was placed in a sucrose-gap chamber in oxygenated Krebs's solution at 30-32°C. The preparation was depolarized by treatment

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TABLE 1. Changes in Inflow of Na^{22} and Ca^{45} into Smooth Muscle Cells under the Influence of AC (M ± 2.5 m)

AC concentration (in g/ml)	Specific activity of strips with Na^{22} (in counts/100 sec/mg)	Specific activity of strips with Ca^{45} (in counts/min/mg)	
	Krebs's solution	Krebs's solution	Sodium-free solution
Control	44,0± 4,2	160,0± 9,2	75,7± 5,0
$1 \cdot 10^{-9}$	—	168,0± 5,0	—
$1 \cdot 10^{-8}$	—	181,3± 5,3*	—
$1 \cdot 10^{-7}$	44,2± 2,8	188,7± 4,2*	—
$1 \cdot 10^{-6}$	45,7± 4,6	144,3± 12,7	81,3± 8,2
$1 \cdot 10^{-5}$	50,0± 4,6*	120,0± 7,1*	97,3± 12,4*
$1 \cdot 10^{-4}$	58,8± 3,7*	99,0± 6,7*	126,0± 12,0*
$1 \cdot 10^{-3}$	65,2± 5,3*	85,7± 5,3*	148,3± 12,4*

*$P < 0.05$ compared with control.

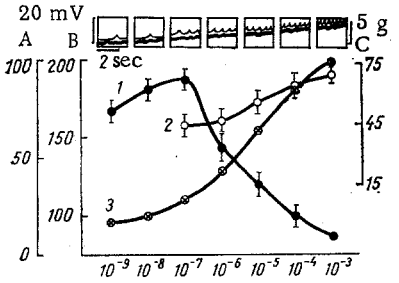

Fig. 1. Effect of different AC concentrations on electrical and mechanical activity and permeability of cell membranes of smooth muscles of guinea pig taenia coli for Ca^{45} and Na^{22} ions. Records of electrical and mechanical activity of smooth-muscle preparations to corresponding AC concentrations are shown on top of figure; middle part shows curves of specific radioactivity of smooth muscles for Ca^{45} (1) and Na^{22} (2), and also magnitudes of coupling (3) (in % of maximum); bottom part shows AC concentrations (in g/ml). Ordinates: A) amplitudes of contractions (in % of maximum), B) specific radioactivity of smooth muscles for Ca^{45} (in counts/min/mg), C) for Na^{22} (in counts/100 sec/mg).

with isotonic potassium sulfate solution with the addition of 200 mg % glucose. The two chambers were separated by a layer of sucrose 1 mm thick. Action potentials (APs) and resting potentials (RPs) were recorded from the chambers by means of chlorided silver electrodes and led to VÉKS-4M dc amplifiers. The tone of the preparation in this case was recorded by a mechanical-electrical transducer. Electrical and mechanical activity were recorded by the photographic recording system of the VÉKS-4M amplifiers. Each series of experiments was performed on six preparations. The results were subjected to statistical analysis [1].

EXPERIMENTAL RESULTS AND DISCUSSION

AC, in concentrations of $1 \cdot 10^{-9}$-$1 \cdot 10^{-3}$ g/ml, caused contraction of the smooth-muscle strips; the effect increased with an increase in concentration (Fig. 1). The electrical activity and tone of the preparations were altered unequally by AC. Changes in tone appeared in response to AC in a concentration of $1 \cdot 10^{-9}$ g/ml, but changes in electrical activity only to higher concentrations (starting from $1 \cdot 10^{-7}$ g/ml). With AC in concentrations of $1 \cdot 10^{-7}$-$1 \cdot 10^{-6}$ g/ml, the frequency of AP generation was increased, but in higher concentrations the increase in frequency of AP generation was negligible and marked depolarization was observed (Fig. 1).

Since contraction of smooth muscles is the result of an increase in the intracellular Ca^{++} concentration [8, 12, 13], changes in the inflow of these ions into the cells depending on the AC concentration were analyzed. AC, in concentrations of $1 \cdot 10^{-9}$-$1 \cdot 10^{-7}$ g/ml, increased the inflow of Ca^{45} (Table 1, Fig. 1); a further increase in the AC concentration led to a decrease in the inflow of isotope into the smooth-muscle cells.

Since changes in electrical activity were virtually absent in AC concentrations of $1 \cdot 10^{-9}$-$1 \cdot 10^{-7}$ g/ml, the most probable factor inducing contraction of the smooth-muscle preparation was the AC-dependent increase in inflow of Ca^{++} ions into the cells. An increase in Na^{22} inflow into the cells was observed in the same AC concentrations ($1 \cdot 10^{-5}$ g/ml and above) in which the specific activity (SA) of the strips with respect to Ca^{45} was reduced. Considering that SA reflects the quantity of isotope in the strip, i.e., the difference between the inward and outward flows, a decrease in SA indicates loss of Ca^{++} ions by the cells. This suggests that the increase in the inflow of Na^{+} ions and the depolarization and increased frequency of spike activity (Fig. 1) are the results of liberation of membrane Ca^{++}.

This view is confirmed by results according to which the permeability of the membrane increases as its Ca^{++} content decreases [7]. Not only the inflow of Na^{22} ions increases, but also the rate of outflow of K^{+} [6] and Ca^{++} [10] ions. Liberation of Ca^{++} ions from the cell membranes of smooth muscles is the trigger mechanism of their contraction [5]. The decrease in the Ca^{45} inflow together with an increase in the rate of Na^{22} inflow can also be explained by competition between Na^{+} and Ca^{++} ions for the ionic channels of the membrane

[4]. In fact, in sodium-free solutions (Table 1), the inflow of Ca^{45} into the cells is increased by the action of AC ($1 \cdot 10^{-5}$-$1 \cdot 10^{-3}$ g/ml).

Under the influence of low AC concentrations ($1 \cdot 10^{-9}$-$1 \cdot 10^{-7}$ g/ml) contraction of smooth-muscle cells is thus determined mainly by an increase in the inflow of Ca^{++} ions into the cells. Under the influence of higher AC concentrations ($1 \cdot 10^{-7}$-$1 \cdot 10^{-5}$ g/ml) liberation of Ca^{++} ions from the membrane becomes the chief component of coupling. This leads to an increase in the sodium permeability of the cell membranes, to the development of depolarization, and to increased frequency of AP generation ($1 \cdot 10^{-5}$-$1 \cdot 10^{-3}$ g/ml). Within certain limits all these coupling components can be superposed.

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