Electrical and Mechanical Events through Activation of Muscarinic Receptor in the Smooth Muscle of Guinea-pig Vas Deferens

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Summary Electrical and mechanical responses of the guinea-pig vas deferens to exogenous acetylcholine (ACh) were observed by means of a single sucrose-gap method. ACh at various concentrations evoked a contraction consisting of two components. The initial component seemed to be independent of a depolarization, while the second one was dependent on it. All ACh responses were abolished by Ca removal from the external solution. The results suggest the existence of a pathway by which ACh even at low concentrations causes a Ca influx without potential changes.

Key Words: guinea-pig vas deferens, acetylcholine, E-C coupling.

Though the role played by endogenous acetylcholine (ACh) in neuromuscular transmission in the guinea-pig vas deferens (SJÖSTRAND, 1981) is not clear, it is well known that exogenously applied ACh evokes a contraction (BIRMINGHAM and WILSON, 1963) and a depolarization (SJÖSTRAND, 1973) through an activation of muscarinic receptors in this tissue. However, there is not sufficient information at present on the relationship between electrical and mechanical responses to exogenous ACh.

For our experiments, a preparation (8 mm in length, 0.6 mm in diameter) isolated from longitudinal smooth muscle layer of the guinea-pig vas deferens was used. The preparation was mounted in a single sucrose-gap apparatus which was essentially similar to that described by STÄMPFLI (1954). One end of the preparation was attached to a strain gauge for isometric tension recording with a resting tension of about 0.5 g. A small portion (2 mm) of the tissue was perfused with a test solution of a constant flow rate. For ACh stimulation, ACh was applied to the tissue for a short period, that is, perfusion of the test solution was stopped for a few seconds to inject 0.025 ml of ACh solution (diluted to re-

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Fig. 1. Electrical and mechanical responses to ACh at various concentrations in the guinea-pig vas deferens recorded with a single sucrose-gap method. (a), (b), and (c) were recorded from the same preparation. The upper tracing of each panel shows the changes in tension, and the lower tracing shows the changes in membrane potential. ACh was applied at bars (see text for the way of application). The concentration of ACh was 10^{-6} M in (a), 10^{-5} M in (b), and 10^{-4} M in (c). Transient downward deflection of potential trace after a force development seems to be an artifact due to some mixture of sucrose with a test solution.

quired concentration with the test solution) into a test solution tube, and then the perfusion was reopened to bring the drug to the tissue. This injection was done far enough from the tissue for full recovery of the tissue and sucrose gap from any damage due to this injecting procedure before the drug reached the tissue. With this short application method, the response to ACh was very reproducible. TTX (10^{-7} M) and guanethidine (10^{-7} M) were employed throughout the experiments to avoid some responses through the nerve system. As seen in Fig. 1, ACh at low concentrations (10^{-6} – 10^{-5} M) evoked a depolarization which was not enough to initiate a development of action potentials. ACh at these concentrations produced a contraction consisting of two components. An initial phasic contraction started just before development of the depolarization, and the time to its peak was faster than that of the depolarization. A second contraction, though its onset was unclear, had a time course similar to that of the depolarization. Therefore the membrane depolarization seems to involve only the second contraction. At a high concentration of ACh (10^{-4} M), the magnitude of the depolarization was increased enough to initiate action potentials, and a large contraction developed

Fig. 2. Effects of removal of external Ca ions on the responses to ACh in the guinea-pig vas deferens. (a), (b), and (c) were recorded from the same preparation but in normal solution containing 2.0 mM CaCl_2 and 1.2 mM MgCl_2 (a), 8 min after exposure to a solution with 6.2 mM MgCl_2 (b), and 15 min after exposure to a Ca-free, 0.5 mM EGTA, and 6.2 mM MgCl_2 containing solution (c). The resting potential was not much affected by the elevation of MgCl_2 concentration, but the membrane was depolarized (about 5 mV) by exposure to the Ca-free solution. The concentration of ACh was 10^{-8} M throughout this experiment.

synchronously to them. At ACh concentrations greater than 10^{-4} M, these components of the contraction overlapped very often owing to a rapid membrane depolarization. All responses to ACh were abolished by treatment with atropine (10^{-6} M).

Another experiment was carried out investigating the effect of Ca-removal from the external solution on the responses to ACh (Fig. 2). Because the concentration of MgCl_2 was elevated from 1.2 to 6.2 mM in a Ca-free solution for a membrane stabilization (BÜLBRING and TOMITA, 1970), the responses to ACh in the test solution but containing 6.2 mM MgCl_2 were firstly observed. The events induced by ACh were not affected with only elevation of MgCl_2 concentration. When secondarily the solution was changed to the Ca-free one, all responses to ACh even at a concentration of 10^{-8} M were abolished.

In the smooth muscle cells, the existence of a pathway by which stimulus substances at large concentrations cause a Ca influx without changes in membrane

potential has been postulated (BOLTON, 1979). More recently, it has been reported that a contraction could be evoked in association with no potential change by noradrenaline (NA) at concentrations which did not produce action potentials in the arteriolar smooth muscle (HIRST and NEILD, 1980). Furthermore in our previous study, very similar phenomena to those in the present study were observed in response to NA in the guinea-pig vas deferens (WAKUI and INOMATA, 1984). The present observations suggest that ACh also may induce a Ca influx without potential changes in addition to voltage dependent Ca influx even at low concentrations in the guinea-pig vas deferens, though a microelectrode method seems to be required to confirm this view.

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