Mechanically induced electrical responses in murine mammary epithelial cells in primary culture

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In mouse mammary epithelial cells in primary culture, mechanical stimulation of a cell induced in other cells within the same colony a short depolarization of less than 15 mV with a duration of 1–8 s and a subsequent, prominent hyperpolarization of 6 mV lasting 10–40 s. Epidermal growth factor induces a spontaneous hyperpolarizing response in cultured mammary cells, and in cells treated with EGF mechanical stimulation produced a greater hyperpolarization, while the amplitude of the depolarizing response was not affected. The amplitude of the mechanically induced hyperpolarization was markedly reduced by quinine and tetraethylammonium, blockers of the Ca^{2+}-dependent K^+ channel. The results suggest that the Ca^{2+}-dependent K^+ channel was involved in the hyperpolarization.

K^+ channel; Hyperpolarization; Membrane potential; (Mammary cell)

1. INTRODUCTION

Electrically non-excitable cells, such as fibroblast L cells [1,2], macrophages [3], erythrocytes [4], and fertilized hamster eggs [5], exhibit rhythmic hyperpolarizing potentials. Mouse mammary epithelial cells in culture, which are also not electrically excitable, show a spontaneous hyperpolarizing response when cultured in the presence of epidermal growth factor (EGF), and produce a depolarizing response when incubated with insulin [6]. These two responses are attributable to the activity of ionic channels, since the membrane conductances increase during the potential changes. The hyperpolarizing response is mediated by activity of a Ca^{2+}-dependent K^+ channel, whereas the ionic species involved in the depolarizing response have not been identified [7].

Earlier studies have shown that a hyperpolarization can be induced mechanically in electrically non-excitable cells [1–3]. To gain further insight into the mechanisms underlying the electrical activity of mammary cells, we examined the effects of mechanical stimulation on cultured mammary epithelial cells. The results were that mechanical stimulation of mammary cells induces a response consisting of an initial depolarization followed by a hyperpolarization involving activity of the Ca^{2+}-dependent K^+ channel.

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2. MATERIALS AND METHODS

The procedures employed for the primary cultures of the mouse mammary epithelial cells using collagen gels have been described elsewhere [8]. The mammary epithelial cells were cultivated for 3–6 days in Medium 199 (Hanks' salts) sup-

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plemented with 5% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37°C with addition of insulin (5 μg/ml) or EGF (50 ng/ml), as indicated. The diameters of the mammary cell in culture were 5–10 μm.

Intracellular recordings of membrane potential and input membrane resistance were made using a conventional high-impedance amplifier with a bridge circuit. The microelectrodes were filled with 2 M potassium citrate and 0.3 M KCl. The reference electrode was an Ag/AgCl/KCl half-cell, in contact with the bath by means of an agar bridge. The recording microelectrode was inserted into the mammary cells in each colony to measure the resting membrane potential and input membrane resistance. After the resting membrane potential had reached a steady level, the upper surface of another cell in the same colony was gently touched for less than 0.5 s with a microcapillary (tip diameter < 1 μm) by hand movement of the manipulator under microscopic observation. A change in the configuration and contrast of the cell surface became apparent. Care was taken not to impale the mammary cell.

For measurements of the effects of drugs, the amplitudes of the electrical responses before and several minutes after addition of drugs were compared. The sources of the chemicals used were as follows: Medium 199 (Hanks' salts) and fetal bovine serum were from Gibco; crystalline pork zinc insulin from Lilly Research Laboratories; mouse submandibular gland EGF from Collaborative Research; quinine hydrochloride and tetraethylammonium chloride from Sigma; Vitrogen 100 from Flow Laboratories. C3H/HeN mice in the 10–12 days of their first pregnancy were obtained from the Animal Breeding Facility, NIH, and reared in our animal room under controlled temperature (25°C) and with lights on between 7:00 a.m. and 7:00 p.m.

3. RESULTS

The resting membrane potentials of mammary cells in culture generally reached a steady level of approx. −20 to −25 mV within several minutes after insertion of a microelectrode (table 1). The peak-to-peak noise of the resting membrane potential varied from 0.5 to 3 mV in the absence of insulin and EGF (fig. 1A,B). Mechanical stimulation of one mammary cell immediately elicited an electrical response in other cells in the same colony. This response consisted of a fast depolarization followed by a slow hyperpolarization (fig. 1A). The depolarization lasted 1–8 s, its amplitude was less than 15 mV, and sometimes it was absent (fig. 1B). The hyperpolarization had a peak height of approx. 6 mV (table 1) and a duration of 10–40 s (fig. 1A,B).

Mammary epithelial cells in culture show spontaneous depolarizing and hyperpolarizing responses in the presence of insulin and EGF, respectively (fig. 1C,D [6]). In mammary cells cultured in the presence of EGF, the amplitude of the mechanically-induced hyperpolarizing response was increased; in contrast, incubation with insulin had no effect (fig. 1C,D; table 1). The peak height of the depolarizing response was unchanged by treatment with EGF or insulin (fig. 1A–D). Repeated stimulation of a cell triggered a similar response (fig. 2) and this could be repeated up to 5 times.

Table 1

Peak heights of the mechanically induced hyperpolarizing response

Culture	Number of observations	Resting membrane potential (mV)	Hyperpolarizing response (mV)
Control	17	24.8 ± 8.1	6.0 ± 8.1
Insulin	6	25.8 ± 7.9	7.5 ± 7.9
EGF	26	20.4 ± 7.2	15.0 ± 5.8[a]

[a] Significantly different from control ($P<0.005$)

Values give an average ± SD. Mammary cells were incubated in the absence of hormones (control) or in the presence of insulin for 2–6 days or in the presence of insulin for 2 days followed by exposure to EGF for 3–48 h

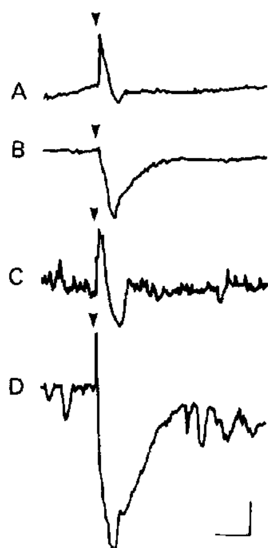

Fig. 1. Electrical response of cultured mammary epithelial cells to mechanical stimulation. Mammary cells were incubated without any hormone and growth factor for 6 days (A,B), or in the presence of insulin for 2 days (C) or in the presence of insulin for 2 days followed by 1 day incubation in the presence of EGF (D). The resting membrane potentials were −30, −20, −33, and −25 mV for A–D. Arrowheads indicate the time of mechanical stimulation. Similar experiments were performed 6–16 times (see table 1). Calibrations, 5 mV and 20 s.

Fig. 2. Electrical response of mammary cells to two successive mechanical stimuli. The same position in the colony was stimulated (arrowheads). Mammary cells were cultured for 2 days in the presence of insulin followed by exposure to EGF for 2 h. Resting membrane potential was −25 mV. The peak heights of electrical responses evoked by successive mechanical stimuli was stable in all preparations tested when the number was 5 or fewer. Calibration bars: 5 mV and 20 s.

The mechanically induced electrical response spread to almost all of the cells in the same colony (>98%) (table 1); it could reach some cells that were located 10–20 cells away from the site of stimulus.

Measurements of the time courses of the membrane potential and input resistance showed that the initial part of the mechanically induced hyperpolarizing response was accompanied by a decrease in the input membrane resistance (fig. 3A–C). However, the input membrane resistance

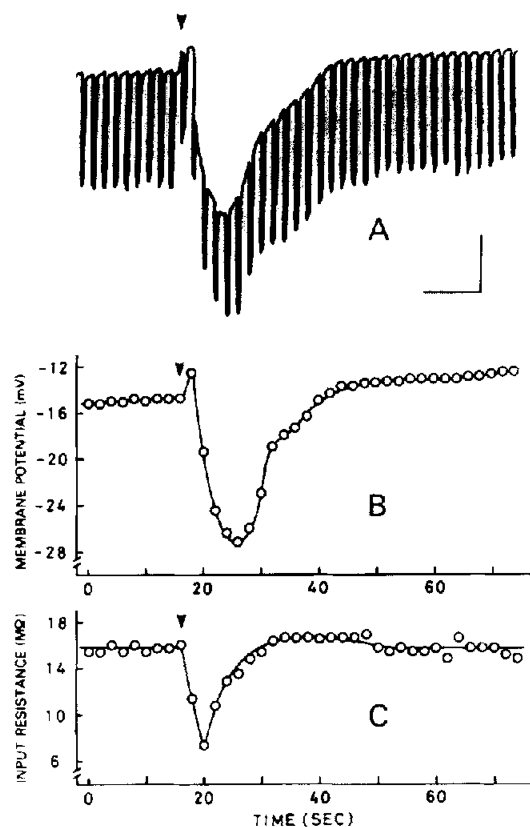

Fig. 3. Time courses of the mechanically induced changes in membrane potential and input resistance. (A) Original trace of the membrane potential and changes produced by injection of a small current pulse (0.65 nA amplitude and 0.5 s duration), (B) membrane potential and (C) input membrane resistance. Arrowheads indicate the time of mechanical stimulation. Mammary cells were incubated for 2 days in the presence of insulin followed by exposure to EGF for 2 days. Similar results were obtained in 6 other experiments. Calibration, 5 mV and 20 s.

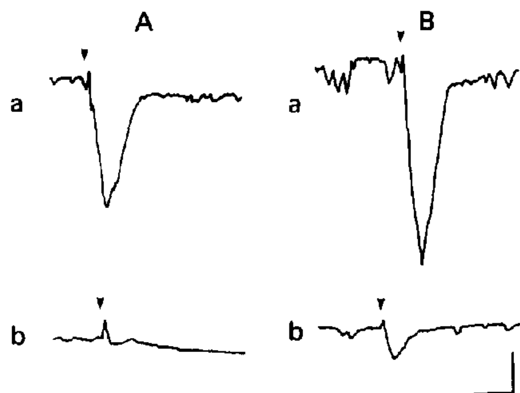

Fig. 4. Effects of quinine and tetraethylammonium on the mechanically induced responses. (A) a, before and b, 3 min after addition of 300 μM quinine. Mammary cells were cultured for 2 days in the presence of insulin followed by incubation in the absence of any hormone for 3 days. The resting membrane potentials were -23 mV for traces a and b. Calibrations, 5 mV and 20 s. Similar results were obtained in 3 other experiments in which the heights of the mechanically induced depolarization varied from 0 to 15 mV. (B) a, before and b, 10 min after addition of 7 mM tetraethylammonium chloride. Mammary cells were cultured for 2 days in the presence of insulin followed by exposure of EGF for 2 days. Resting membrane potential (-15 mV) remained unchanged. Calibration bars: 5 mV and 20 s.

returned to its initial value faster than did the membrane potential.

Our previous studies showed that EGF-induced hyperpolarizing response was inhibited by blockers of the Ca^{2+}-dependent K^+ channel, quinine and tetraethylammonium [7]. Quinine markedly decreased the peak height of the hyperpolarization of the mechanically induced response but had no effect on the depolarizing phase (fig. 4A). Tetraethylammonium (7 mM) also greatly reduced the peak height of the hyperpolarizing phase (fig. 4B). These results suggested that activity of the Ca^{2+}-dependent K^+ channel was important for the mechanical activation of hyperpolarization.

4. DISCUSSION

Here, mechanical stimulation was shown to induce a membrane response consisting of an initial, short depolarization and a subsequent, prominent hyperpolarization in mammary cells in culture. Previously, we showed that mammary cells exhibit spontaneous hyperpolarizing potentials in the presence of EGF [6]. In the present study, the effects of EGF and mechanical stimuli were found to be synergistic (fig. 1D). Activity of a Ca^{2+}-dependent K^+ channel appears to be important for the mechanically induced hyperpolarizing response, as is also true for the EGF-induced spontaneous hyperpolarizing response [7]. The amplitude of the depolarization phase of the mechanical response varied from preparation to preparation more than did the hyperpolarization. The greater variation in size of the depolarization phase might be due to its having a greater voltage dependence. Patch-clamp experiments should reveal whether this is, in fact, correct.

In the mechanically activated response, the membrane input resistance returned to its resting level faster than did the membrane potential. This difference could be explained if mechanical stimulation also activated the electrogenic Na^+/K^+ pump, which participates in the hyperpolarization without much change in the input membrane resistance. It is unlikely that the differential recoveries of the membrane potential and the input resistance were due to the effect of the membrane capacitance on the recovery of the membrane potential, since the membrane time constant of the mammary cells, less than 5 ms, was too small to influence the time of recovery of the hyperpolarization [6].

It has been proposed that mechanical stimulation of the plasma membrane of fibroblast L cells activates Ca^{2+} channels and increases the cytoplasmic Ca^{2+} concentration [9]. This, in turn, activates the Ca^{2+}-dependent K^+ channel and provokes a hyperpolarization. The increased level of intracellular Ca^{2+} activates the Ca^{2+} pump, and thus lowers intracellular Ca^{2+}. This will terminate the hyperpolarization. A similar proposal can be made to explain the mechanically activated response of mammary cells. At present, however, the molecular nature of the initial events responsible for the transduction of the mechanical stimulus is not known.

Several investigators have proposed that the physiological role of the hyperpolarizing response in fibroblast L cells and macrophages may be related to contractile systems, such as phagocytosis and pinocytosis [3,9,10]. The physiological

significance of the mechanically responsive electrical activity in mammary cells is unclear at the present time, although these cells are subject to mechanical stimuli by adjacent cells during the period of cell growth and milk secretion. The present findings may be useful for studying the regulation and function of electrical activity of the mammary cell membrane.

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