Involvement of the Ca^{2+}-dependent K^+ channel activity in the hyperpolarizing response induced by epidermal growth factor in mammary epithelial cells

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Epidermal growth factor (EGF) induces a hyperpolarizing response of 5–20 mV amplitude in mouse mammary epithelial cells in culture. The amplitude of the hyperpolarizing response was reduced by more than 60% within several minutes after addition of blockers of voltage and/or Ca^{2+}-dependent K^+ channels such as tetraethylammonium (7 mM) or quinine (0.29 mM). Both nifedipine (0.15 mM), a blocker of the Ca^{2+} channel, and ruthenium red (2 mM), an inhibitor of the Ca^{2+}-binding site, also reduced the amplitude of the hyperpolarizing response by more than 60%. The Ca^{2+} ionophore, A23187 (3.8 μM), induced a large hyperpolarization, which was 25–40 mV and lasted about 3 min. These data suggest that activity of the Ca^{2+}-dependent K^+ channel was involved in the EGF-induced hyperpolarizing response of the mammary epithelial cells.

(Mammary epithelial cell) Epidermal growth factor Ca^{2+} activation K^+ channel Membrane potential Hyperpolarization

1. INTRODUCTION

Epidermal growth factor (EGF), a polypeptide hormone consisting of 53 amino acid residues [1], is involved in the growth and differentiation of mouse mammary epithelial cells in vivo and in vitro [2,3]. Recent measurements of the membrane potentials of mammary epithelial cells with microelectrodes have shown that EGF induces spontaneous hyperpolarizing responses which are about 5–20 mV and last up to 10 s [4]. The hyperpolarizing response may be closely related to the mechanisms responsible for the mitogenic action of EGF.

The hyperpolarizing response was attributed to an opening of a K^+ channel since it had a null potential of -90 to -100 mV [4]. To learn more about the hyperpolarizing response, we used intracellular recording techniques to investigate the effects on this response of blockers of Ca^{2+}-dependent K^+ channels, a Ca^{2+}-channel blocker and an inhibitor of Ca^{2+}-binding sites. The results suggest that the hyperpolarizing response is produced by a Ca^{2+}-activated K^+ current.

2. MATERIALS AND METHODS

The primary culture of mammary epithelial cells was performed according to standard protocols described in [2,3]. Membrane potentials were measured using a conventional high-impedance amplifier with a bridge circuit for current injection [4].

Several minutes after impalement of the mam-

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mary cell, the resting membrane potential reached the steady level of approx. −30 mV. After the steady potential was reached, a drug solution was added to the culture dish. The magnitudes of the hyperpolarizing responses and resting membrane potentials before and several minutes after the addition of the drug were compared. Ethanol and dimethyl sulfoxide (DMSO) were used as vehicles for the addition of nifedipine and A23187, respectively. Final concentrations of ethanol and DMSO were 1–2 and 0.1%, respectively, and these levels of ethanol or DMSO alone had no effect on the resting membrane potential or the electrical activities of the mammary cells. The other drugs were dissolved in the medium.

The sources of the chemicals were as follows: medium 199 (Hanks' salt) from Gibco; bovine serum albumin from Miles-Yeda; insulin from Lilly Research Laboratories; EGF from Collaborative Research; tetraethylammonium chloride, quinine hydrochloride, nifedipine and ruthenium red from Sigma; A23187 from Calbiochem-Behring; Vitrogen 100 (purified collagen) from Flow Laboratories. Other chemicals were purchased from commercial suppliers. C3H/HeN mice in the 10–12th day of their first pregnancy were obtained from the Animal Breeding Facility, NIH.

3. RESULTS

The average resting membrane potential of the mammary cells cultured without hormones was approx. −30 mV (fig.1A). The potential was generally quite stable and the peak-to-peak noise was of the order of 1 mV. The cultivation of mammary cells in the presence of EGF for 2 days induced spontaneous hyperpolarizing responses of approx. 5–20 mV peak amplitude which lasted as long as 10 s (fig.1B).

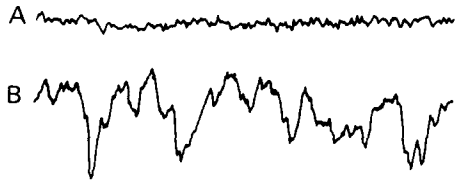

Fig.1. Induction of the spontaneous hyperpolarizing response by EGF. Mammary epithelial cells were cultivated for 2 days in the absence (A) or presence of EGF (50 ng/ml) (B). Resting membrane potential was −30 mV for A and B. Calibration bars: 5 mV and 20 s.

The effects of tetraethylammonium and quinine, which block voltage-dependent and/or Ca^{2+}-activated K^+ currents [5,6], on the hyperpolarizing response were measured. 3–10 min after addition of these drugs, the amplitude of the hyperpolarizing response decreased to less than 60% of the control value (figs 2 and 3).

Both nifedipine, a blocker of Ca^{2+} channels [7] and ruthenium red, an inhibitor of Ca^{2+}-binding sites [8], also reduced the amplitude of the hyperpolarizing response by a similar extent (figs 4 and 5). Except for quinine, these drugs had little or no effect on the resting membrane potential. In about 60% of the cells, addition of quinine caused an acute depolarization of approx. 10 mV.

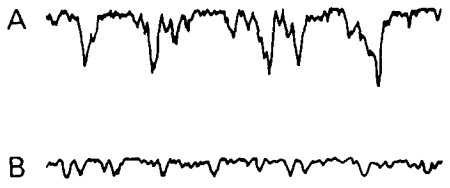

Fig.2. Effects of tetraethylammonium on hyperpolarizing response. Mammary epithelial cells were cultivated for 3 days in the presence of EGF (A) and then treated with 7 mM tetraethylammonium for 10 min (B). Resting membrane potential (−15 mV) remained unchanged. Similar results were obtained in 4 other cells where the concentration of tetraethylammonium ranged from 7 to 10 mM. Calibration bars: 5 mV and 20 s.

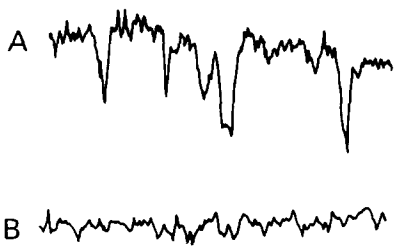

Fig.3. Effects of quinine on the hyperpolarizing response. Mammary epithelial cells were cultivated for 3 days in the presence of EGF (A) and then treated with 0.29 mM quinone for 3 min (B). The resting membrane potential (−22 mV) remained unchanged. Similar results were obtained in 5 other cells where the concentration of quinine ranged from 0.1 to 0.3 mM. Calibration bars: 5 mV and 20 s.

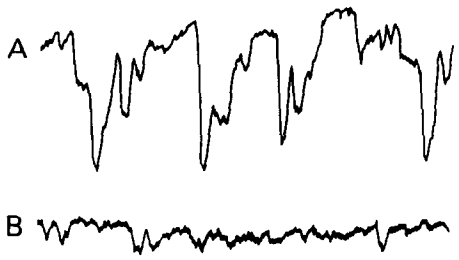

Fig.4. Effects of nifedipine on the hyperpolarizing response. Mammary epithelial cells were cultivated for 3 days in the presence of EGF (A) and then treated with 0.15 mM nifedipine for 20 min (B). Resting membrane potential (−30 mV) remained unchanged. Similar results were obtained in 9 other cells where the concentration of nifedipine ranged from 0.1 to 0.2 mM. Calibration bars: 5 mV and 20 s.

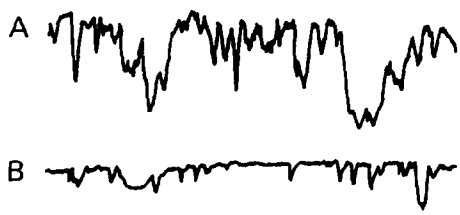

Fig.5. Effects of ruthenium red on the hyperpolarizing response. Mammary epithelial cells were cultivated for 3 days in the presence of EGF (A) and then treated with 2 mM ruthenium red for 4 min (B). Resting membrane potential (−30 mV) remained unchanged. Similar results were obtained in 3 other cells. Calibration bars: 5 mV and 20 s.

Fig.6. Effect of Ca^{2+} ionophore A23187 on the membrane potential. Mammary epithelial cells were cultured in the presence of EGF for 1 day, and then treated with A23187 (3.7 μM) at the time indicated by the arrow. The resting membrane potential was −22 mV. Similar results were obtained in 6 other cells. Calibration bars: 5 mV and 20 s.

These results suggested that a Ca^{2+}-activated K^+ current may have been responsible for the hyperpolarizing responses. Therefore, the effects of increased cytoplasmic Ca^{2+} levels were investigated. Addition of A23187 (3.8 μM), a Ca^{2+} ionophore [9], produced a large hyperpolarization of the mammary cells (fig.6). The amplitude of this response was about 25–40 mV and lasted about 3 min.

4. DISCUSSION

We reported previously that EGF induced spontaneous hyperpolarizing responses in cultured mammary epithelial cells [4]. This electrical phenomenon was attributed to activation of K^+ channels, since the peak height of the response was reduced by hyperpolarization, the null potential of the hyperpolarizing response was estimated to be between −90 and −100 mV, and the input membrane resistance decreased during the response [4]. The reduction in the amplitude of the hyperpolarizing response by tetraethylammonium and quinine (figs 2 and 3) suggests that activity of the Ca^{2+}-dependent K^+ channel was involved in the hyperpolarizing response. The hypothesis that an increase in the intracellular Ca^{2+} concentration was critical to the triggering of the hyperpolarizing response was supported by the finding that both nifedipine and ruthenium red reduced the size of the hyperpolarizing response (figs 4 and 5); in addition, a large hyperpolarization was induced by a Ca^{2+} ionophore, A23187 (fig.6). Further, EGF has been shown to increase the cytoplasmic Ca^{2+} concentration in its target cells [10]. Based on these observations, we postulate that EGF induces the hyperpolarizing response in mammary cells by stimulating the activity of Ca^{2+}-dependent K^+ channels itself or by Ca^{2+} entry or mobilization.

Similar spontaneous hyperpolarizing responses have been found in various other cells such as macrophages [11], fibroblastic L cells [12], sympathetic ganglion cells [13] and pancreatic β-cells [14]. The activity of the Ca^{2+}-dependent K^+ channel has been implicated in these responses because they are sensitive to blockers of the Ca^{2+}-dependent K^+ channels such as tetraethylammonium [5,12,15] and quinine [14,16]. Moreover, perfusion of A23187 causes large hyperpolarizations in some of these cells [11,12]. However, these

responses are apparently not dependent on any growth factors and their functional roles remain unclear.

In mammary cells, EGF induces the hyperpolarizing response prior to cell proliferation [4]. Our preliminary experiments show that quinine blocks EGF stimulation of mammary cell proliferation, suggesting that the hyperpolarizing response may play an important role in cell growth. To determine whether this is indeed the case, experiments are in progress to assess this quinine response and to determine whether alterations in the intracellular Ca^{2+} concentration and the activity of Ca^{2+}-dependent K^+ channels play a role in the mitogenic action of EGF in mammary cells.

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