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An upward shift of intracellular pH rather than the final absolute pH value is critical for controlling gap junction permeability in tumor promoter-treated cells

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The tumor promoter TPA is found to inhibit gap junction permeability in monolayer cultures of hamster fibroblasts. This effect is associated with an increase in intracellular pH. Here we show that neither an increase in pH_i alone nor TPA treatment under conditions preventing pH_i-shift affect gap junction permeability. It is not the level of pH_i reached, but rather the pH_i-shift itself that is essential for the inhibition of gap junction permeability in the presence of TPA.

Cell–cell communication; Intracellular pH

1. INTRODUCTION

Small changes in intracellular pH have been shown to be important in cell physiology [1–3]. It was established that in some cases the upward shift of intracellular pH, pH_i, by 0.2–0.3 units is required for cell stimulation by various growth facors and tumor promoters such as TPA [3]. However, different pH_i levels are reported by different authors.

In the present work using a new experimental system of pH-controlled TPA-mediated gap junction closure we demonstrate that it is the small pH_i-shift itself, rather than the actual level of pH_i reached that is essential.

2. MATERIALS AND METHODS

We have used dense monolayers of hamster fibroblasts DM-15. Confluent cell monolayers were obtained as described earlier [4]. Before the experiment, the medium was changed for one with high K^+ consisting of 130 mM KCl, 5 mM NaCl, 10 mM glucose, buffered with 10 mM Hepes. Intracellular pH was evaluated in individual cells in cultures stained with BCECF by measuring the ratio of fluorescence, excited at two wavelengths with interference reflection filters and a photomultiplier-equipped Zeiss Microscope as described in [5]. Calibration curves were obtained according to [6]. We have checked that the high-K^+ medium itself does not affect gap junction permeability.

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Abbreviations: BCECF, bis(carboxyethyl)-5(6)carboxyfluorescein; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PKC, protein kinase C

Gap junction permeability was evaluated by a standard method of injecting Lucifer yellow into one of the cells and counting the number of surrounding cells starting to fluoresce due to the intercellular dye transfer. In each experiment 10–20 cells were injected with Lucifer yellow and the number of the stained neighboring cells was measured. To equilibrate the proton concentration between the outer medium and the cytoplasm nigericin (Sigma) from stock solution in ethanol was added to the cells at a concentration of 10^{-5} M. pH_i-shift was achieved by shifting the cultures from the nigericin-containing high-K^+ medium at one pH to a similar medium at another pH. TPA in the concentration of 2×10^{-7} M was added to the cultures 10 min prior to the pH_i shift.

3. RESULTS AND DISCUSSION

Gap junction-mediated cell–cell communication is regarded as an important factor controlling the development and differentiation of various tissues [7,8]. Tumor promoters, including TPA, inhibit gap junction permeability [9–11]. Simultaneously TPA increased pH_i by 0.1–0.3 units (both effects are presumably mediated by activation of PKC which phosphorylates gap-junction proteins [12,13] and membrane antiporters [14,15]). To find out whether the increase of pH_i and inhibition of gap junction permeability are related to each other, we developed an experimental system where pH_i can be shifted without TPA, and where, on the other hand, TPA-induced pH_i-shift can be prevented.

We experimentally stabilized the pH_i of cell cultures at the desired level using the exogenous K^+/H^+ antiporter nigericin. In high-K^+ medium where the concentration of K^+ inside the cell is equal to that outside, nigericin equilibrates the concentration of protons bringing the pH_i to be equal to the pH of the outer

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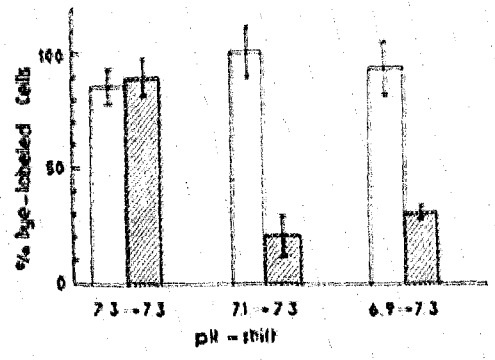

Fig. 1. A shift of intracellular pH is necessary for the inhibition of gap junction permeability by TPA. Empty columns, control (no TPA); hatched columns, TPA-treated cells. Vertical axis: the percentage of neighboring cells stained by Lucifer yellow after the dye was injected into one of them. (The number of the cells stained in non-treated cultures in full serum-supplemented media is taken to be 100%.) Each column presents the mean ± SE of 2–3 experiments.

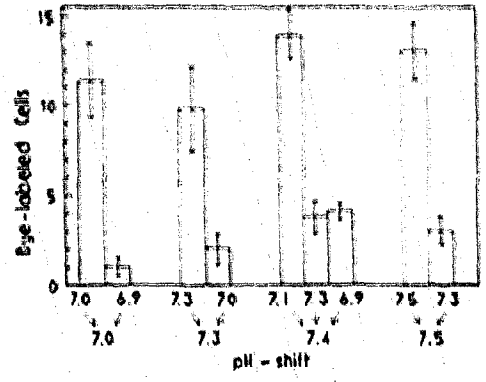

Fig. 2. Inhibition of the gap junction permeability in the presence of TPA is independent of actual level of pH_i but requires the pH_i-shifts. The columns represent the mean ± SE of Lucifer yellow-stained cells surrounding the central one in which the dye was injected. The experiments were performed as described in the legend to Fig. 1, but high-K^+ media at various pHs were used. Simultaneously, with application of TPA, pH_i was shifted and the gap junction permeability was evaluated. The initial and the final levels of pH_i are indicated below the columns. Note that the gap junction permeability at any pH_i level depends on whether pH_i remained constant (left column in each group) or was shifted from the lower level.

medium [6]. (High-K^+ medium neither affects gap junction permeability in control cells, nor does it interfere with the inhibitory effect of TPA on gap junction. TPA inhibits gap junction permeability in high-K^+ serum-free medium by 60–70% as it does in full serum-supplemented media. In both media after TPA treatment (2×10^{-7} M, 10 min) pH_i increased by 0.1–0.3 units.)

TPA has no effect on gap junction permeability if by incubation in high-K^+ medium with nigericin pH_i was fixed at a certain level between 7.0 and 7.5. The pH_i-shift alone had no effect on gap junction permeability without TPA (Fig. 1). Gap junction permeability was inhibited only if intracellular pH was shifted up by 0.1–0.3 units simultaneously with TPA treatment (by transferring the cultures from one nigericin-containing high-K^+ medium at one pH level to a similar medium but at higher pH) (Fig. 1). Thus an upward pH_i-shift is necessary for TPA-induced inhibition of gap junction permeability.

The question arises as to whether there is a threshold of pH_i above which TPA inhibits gap junction permeability. In an attempt to answer this question we shifted pH_i of TPA-treated cells 0.1–0.5 units beginning from different initial pH_is between 6.9 and 7.3. All of these shifts allowed inhibition of intercellular dye transfer by TPA (Fig. 2). In contrast, if pH_i was stabilized and no shift of pH_i was allowed to occur, TPA did not inhibit gap junction permeability.

Based on the results of the experiments described in the present work, it can be assumed that in addition to the direct action of PKC on gap junctions, there is another controlling mechanism mediated by an upward shift of pH_i. The final result, that is the inhibition of gap junction permeability, is achieved if and only if both mechanisms are activated.

It is the change in pH_i rather than an upward shift to a particular level of pH_i that is required for TPA to inhibit gap junction permeability. It may be that there are molecules whose activity is changed by relative protonation or deprotonation rather than by their absolute state. Alternatively, a decrease in proton concentration in the cytoplasm changes the proton gradients across some intracellular membranes. Enzymatic reactions driven by transmembrane proton gradients have been described [16].

pH_i-shifts, rather than the actual level of pH_i, may be essential for other cellular functions where the involvement of small changes in pH_i have been reported [17–19].

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