Changes in Cell Morphology and Cell-to-cell Adhesion Induced by Extracellular Ca\(^{2+}\) in Cultured Taste Bud Cells

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Note

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Cell morphology and cell-to-cell adhesion of taste bud cells were significantly altered by extracellular Ca\(^{2+}\) during in vitro culture. Under high Ca\(^{2+}\) condition (above 0.5 mM), the cells were tightly associated with each other and formed packed aggregates. Under low Ca\(^{2+}\) condition (below 0.1 mM), the cells were dispersed and had an elongated shape. These two forms were reversible and specifically dependent on Ca\(^{2+}\). The results indicate that extracellular Ca\(^{2+}\) regulates cell shape and cell-to-cell adhesion of taste bud cells.

Key words: taste bud cells; extracellular Ca\(^{2+}\); cell morphology; cell-to-cell adhesion; in vitro culture

Taste buds contain different types of cells.\(^1\) The taste bud cells are epithelial cell lineage, turn over every 10 days, and appear to be derived from specific stem cells residing in or around the taste bud.\(^1\)–\(^4\) Physiology and function of the cells are unique in many respects such as cell morphology, membrane potential, neurotransmitter release, differentiation and apoptosis.\(^1\)–\(^5\) However, several difficulties stand in the way of analyzing them at cellular and molecular levels. One of them might be the lack of in vitro culture system in which physiological functions are preserved. We have recently reported a standard method for primary culture of taste buds, where the cells maintain viability over 5 d and retain several taste-bud-specific molecular markers.\(^5\) During the course of the optimization of this system, we noticed that extracellular Ca\(^{2+}\), which was optimized as 0.15 mM under the standard condition, is critical for cell morphology and attachment onto the culture dishes. In this paper, we examined the effects of Ca\(^{2+}\) on taste bud cells in vitro.

Taste buds of circumvallate papillae were isolated from rat tongue epithelia as described previously.\(^8\) Fifty to 100 taste buds were placed on a 35-mm culture dish which was coated with Matrigel, and cultured in mKGM medium consisting of 17.6 g/l MCDB153, 1.2 g/l NaHCO\(_3\), 5 mg/l insulin, 14.1 mg/l α-phosphorylethanolamine, 10 μg/l epidermal growth factor, 100 U/l transferrin, 0.5 mg/l hydrocortisone, 10 mg/l streptomycin, 2 ml/l Matrigel, 50 mg/l bovine pituitary extract, and 0.03 to 1.5 mM CaCl\(_2\). Cell morphology at different Ca\(^{2+}\) concentrations were observed at intervals under an inverted microscope equipped with a phase contrast optic system. Viability was maintained at least for 3 d from the initiation of culture (data not shown) as described previously.

As shown in Fig. 1, the appearance of the cultured cells was significantly different and dependent on Ca\(^{2+}\) concentration. Under low Ca\(^{2+}\) condition (0.03 mM), the cells were elongated and dispersed onto the dish (Fig. 1(A)). Under intermediate Ca\(^{2+}\) condition (0.15 mM), most cells were aggregated and some cells around the aggregates were elongated (Fig. 1(B)), which was the same as our previous observation.\(^8\) Under high Ca\(^{2+}\) condition (1.5 mM), total cells were aggregated to form a mass, probably corresponding to the initial taste bud, and the cell shape was apparently thin and elongated (Fig. 1(C)). These profiles indicate that the extracellular Ca\(^{2+}\) promotes cell-to-cell contact. In addition, cell adhesion might be also influenced by Ca\(^{2+}\), because the number of the cells retained under low Ca\(^{2+}\) condition was smaller than those under higher Ca\(^{2+}\) conditions.

Next, we examined the initial stage of these phenomena. In the early period of the culture, up to 6 h, no significant or only a slight difference was observed (Figs. 2(B), (F) and (J)); aggregates were seen, constituted with round cells that were probably derived from single taste buds. After 12 h under low and...
intermediate Ca²⁺ conditions, some cells began to 
elongate around the aggregates (Figs. 2(C) and (G)). 
After 24 h, most of the cells at low Ca²⁺ were dis-
persed and took an elongated form, although a part 
of the cells had detached from the culture dish to be a 
round shape, and as a result the cell number 
decreased with time (Fig. 2(D)). In intermediate 
Ca²⁺, most cells still constituted loose aggregates and 
some were dispersed (Fig. 2(H)). On the other hand, 
at high Ca²⁺, the cells still formed tight aggregates 
similar to the initial stage (Figs. 2(K) and (L)). These 
results indicate that the changes in the cell aggrega-
tion and cell shape occur gradually. Low recovery of 
the cells observed in low Ca²⁺ culture (Fig. 1(A)) 
might be explained by the detachment, suggesting 
that cell-to-matrix adhesion is not enough under the 
low Ca²⁺ condition.

Drastic Ca²⁺-dependent changes of the cultured 
taste bud cells were slow and needed about a half day 
after isolation of taste buds as shown above. 
However, the period included the process of adapta-
tion into in vitro culture and might not directly reflect

Fig. 1. Cell Morphology and Aggregation of Taste Bud Cells. 
Taste buds were cultured in mKGM medium containing 0.03 mM (A), 0.15 mM (B), and 1.5 mM (C) Ca²⁺ for 3 d.

Fig. 2. Initial Process of Ca²⁺-dependent Changes in Cell Morphology and Adhesion. 
Taste buds were isolated and cultured in mKGM medium containing 0.03 mM ((A)-(D)), 0.15 mM ((E)-(H)), or 1.5 mM ((I)-(L)) Ca²⁺. 
Photographs were taken immediately after placing the taste bud cells onto the culture dishes ((A), (E), and (I)), after 6 h ((B), (F), and 
(J)), after 12 h ((C), (G), and (K)), and after 24 h ((D), (H), and (L)). The same areas are shown in each series.
Effects of Ca\(^{2+}\)-transition on Cell Morphology and Adhesion.

Taste buds were isolated and cultured for 24 h in mKGM medium containing either 0.03 mM ((A)–(E)) or 1.5 mM ((F)–(J)) Ca\(^{2+}\). After that, Ca\(^{2+}\) concentration was changed to 1.5 mM ((A)–(E)) or 0.03 mM ((F)–(J)). Photographs were taken immediately after the change ((A) and (F)), after 4 h ((B) and (G)), after 8 h ((C) and (H)), after 12 h ((D) and (I)), and after 24 h ((E) and (J)). The same cells are shown in each series.

The time course of cellular response to Ca\(^{2+}\). We then examined the response induced by a shift of Ca\(^{2+}\) concentration during the culture, which would also show the reversibility of the changes. First, the taste bud cells were cultured under either low or high Ca\(^{2+}\) condition for 12 or 24 h. After that, the cells were transferred either from low Ca\(^{2+}\) to high Ca\(^{2+}\) or from high Ca\(^{2+}\) to low Ca\(^{2+}\), and the time course of the cell aspects were observed. Figure 3 shows the results of the Ca\(^{2+}\)-shift after the 24-h culture. The Ca\(^{2+}\) change from low to high concentration induced cell aggregation and morphological changes in 4 h (Fig. 3(B)), and after 8 h in high Ca\(^{2+}\), most cells formed aggregates (Fig. 3(C)). After 24 h, cell masses were observed (Fig. 3(E)), which were similar to the cases of the continuous high Ca\(^{2+}\) condition (Figs. 1(C) and 2(L)). On the other hand, the Ca\(^{2+}\) change from high to low also induced rapid response, which was the reverse of that from low to high (Figs. 3(F)–(J)); the cells began to disperse in 4 h (Fig. 3(G)), and most cells were elongated and could be individually identified in 12–24 h (Figs. 3(I) and (J)). At the same time, some of the cells became round and were floating from the dish as in the case under continuous low Ca\(^{2+}\) condition (Figs. 1(A) and 2(D)). These observations show that the Ca\(^{2+}\)-dependent changes in the cell morphology and adhesion occur rapidly and are reversible.

Finally, we checked the specificity of ions. Using the above assay method, we replaced Ca\(^{2+}\) with another divalent cation. If an ion could substitute Ca\(^{2+}\), cell aggregates preserved by the activity of Ca\(^{2+}\) would be maintained when changes from 1.5 mM Ca\(^{2+}\) into 0.03 mM Ca\(^{2+}\) added with the ion. That is, differences in morphology and aggregation between low Ca\(^{2+}\) only and low Ca\(^{2+}\) supplemented with another cation would show the effect(s) of the ion. We selected 6 divalent cations: Mg\(^{2+}\), Mn\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Ba\(^{2+}\), and Co\(^{2+}\). Among them, Cu\(^{2+}\) and Co\(^{2+}\) were highly toxic and most cells floated within 3 h (data not shown). As shown in Fig. 4, the other 4 ions were shown to be unable to substitute Ca\(^{2+}\); the cells began to disperse in the medium containing each 1.5 mM cation in the same way as those in low Ca\(^{2+}\), although the frequencies of the elongated cells were lower when each of Mn\(^{2+}\), Ba\(^{2+}\), and Ni\(^{2+}\) was added than that lacking these supplementary ions, probably due to the moderate toxicity of the ions.

This study showed the drastic and specific effects of Ca\(^{2+}\) on the morphology and cell-to-cell adhesion of taste bud cells in vitro. Although it is known that Ca\(^{2+}\) has significant effects on cells including morphology, those observed here are extremely strong and suggest specific physiology of the taste bud cells. Since taste bud cells are spindle-shaped and packed into the special structure (i.e. taste bud) in vivo, it is probable that they have strong cell-to-cell adhesion, junctions, and communication in addition to specific intracellular structures of cytoskeletons, all of which are known to be influenced by Ca\(^{2+}\). Thus, the observations shown here might be directly related to cellular functions and structures of the taste bud cells. Although target molecules by Ca\(^{2+}\) have not yet been identified, several molecules or pathways can be considered. Cadherins, intercellular homotalic adhesion molecules forming adherens junctions, are regulated by extracellular Ca\(^{2+}\). Tight junctions are also regulated by extracellular Ca\(^{2+}\). GAP junctions formed by connexins are also reported to be influenced by Ca\(^{2+}\), which is further linked to the tight junction via the interaction between a connexin and ZO-1. In addition, it was reported that extracellular Ca\(^{2+}\) regulates cell morphology by way of the arachidonic acid cascade. Matrix metalloproteinases might be also involved in the cell-to-cell and/or cell-to-matrix interaction. Taken together, there are many pathways dependent on extracellular...
Ca²⁺, which might result in cytoskeletal changes in addition to those directly in cell-to-cell adhesion, finally to reach cellular morphology and functions. These specific profiles and mechanisms including molecular components involved will be a clue to understand the physiology and functions of the taste bud.

References