NATURE FOOD SCIENCE
Vol. 85, opus 3, pp. 38-486, April 1, 1998
(Received for publication March 25, 1998)

Apoptartsis, a novel cell death mechanism caused by glycosylation with cheeriose residues

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The signal transduction mechanisms that control taste recognition are mediated by cell-cell contact in the taste bud. Inappropriate activation of taste recognition can abrogate the ability to differentiate between toxic and non-toxic foods. We describe the identification of a rapid, novel cell death mechanism, apoptartsis, that prevents the mistaken taste identification of American cheese as expensive Swiss Gruyère. Glycosidation studies reveal that apoptartsis involves a change in the basil levels with thyme of the protein cumin, and is caused by the addition of seven cheeriose molecules to amino acid residue 32, which is fennelalanine in normal humans. Mutation of fennelalanine 32 to an ovaltine residue leads to the appearance of a series of partially homologous proteins including SADD, BADD, GLADD, and MADD. Furthermore, we show that the onset of apoptartsis is preceded by the interaction of cumin with a previously unidentified protein, BEAN15.

Taste recognition in humans has only recently begun to be understood on a molecular level. The identification of a mutation causing taste deficiency (McDonald and Sanders, 1994) has led to cloning and analysis of genes encoding 31 taste recognition proteins (Baskin and Robbins, 1997). An effective assay for these proteins has recently been described that evaluates individuals' taste for Green-Fluorescent-Protein-expressing chicken egg with heat denatured porcine skeletal muscle tissue (Seuss *et al*., 1995).

It was initially thought that the primary mediator of taste was the heterodimer of the proteins republicin and democratin, which inhibited the expression of a class of taste recognition genes transcribed by RNA polymerase class IV (Pol ITICS), because republicin-democratin double transgenic mice exhibited absolutely no taste (Gingrich *et al*., 1994). However, we show here that this observed effect is an artifact of the cultured cell line used for the previous studies, as our findings show that expression of Pol ITICS-transcribed genes do not support culture, and, through a complex signal transduction cascade, actually lead to the inhibition of taste. We propose instead that taste recognition in the processed American cheese model (Kraft, 1995) is mediated by cell-cell contact leading to activation of BADD, and that the novel process of apoptartsis prevents inappropriate BADD taste.

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MATERIALS AND METHODS

Isolation of republicin and democratin

The republicin-enriched protein fraction was purified from total banana protein extract as described previously (Dole *et al*., 1996), and republicin was purified on Sepharose beads covalently linked to an IgY antibody from a quayle egg system (ReaGen Corp., Washington, DC). For democratin purification, recombinant clintin including the leader peptide was produced in *E. coli* and collected by dialysis. Subsequent enzymatic Trippsin cleavage (Starr and Lewinsky, 1998) yielded dilute democratin, which was concentrated by centrifugation through a Centristcon filter.

Glycosidation studies

Taste bud protein extracts (Jagger and Richards, 1972) were immunoprecipitated using undergraduate-derived antibodies against spice proteins, separated on a non-denaturing gel (Jello Co., Gainsville, FL), and identified with a goat-anti-undergraduate secondary antibody (Stanford University Work Study program, Stanford, CA). As a positive control, cheeriose was purified as previously described (Kellogg *et al*., 1989), and cereal dilutions were made to ensure that the concentration would be within detectable range. A cocktail of protease inhibitors (Sigma, St. Louis, MO) and a cocktail of fruit (Del Monte, Huntington, AL) were diluted 1:1 with 2x H_2O, and added to all samples at 1:100 dilution. Samples were incubated at 37°C for 1 hr, during which time 100 mg caffeine was administered to the investigators. For glycosidation, 250 mM EDTA was