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Microwave processing of gustatory tissues for immunohistochemistry

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HIGHLIGHTS

- Microwave fixation of gustatory tissue improves fixation quality.
- Microwave processing decreases the time necessary to complete experimental procedures.
- Antigen retrieval is not needed when using a microwave processor.
- Image quality from the microwave processor is superior to conventional methods.

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ABSTRACT

We use immunohistochemistry to study taste cell structure and function as a means to elucidate how taste receptor cells communicate with nerve fibers and adjacent taste cells. This conventional method, however, is time consuming. In the present study we used taste buds from rat circumvallate papillae to compare conventional immunohistochemical tissue processing with microwave processing for the colocalization of several biochemical pathway markers (PLCβ2, syntaxin-1, IP_3R3, α-gustducin) and the nuclear stain, Sytox. The results of our study indicate that in microwave versus conventional immunocytochemistry: (1) fixation quality is improved; (2) the amount of time necessary for processing tissue is decreased; (3) antigen retrieval is no longer needed; (4) image quality is superior. In sum, microwave tissue processing of gustatory tissues is faster and superior to conventional immunohistochemical tissue processing for many applications.

1. Introduction

Combining a fluorescent dye with an antibody was first performed in 1941 (Coons et al., 1941). Since then, the technique of immunohistochemistry has been carried out using various fixatives and protocols (Giberson and Demaree, 2001; Galvez et al., 2006; Ferris et al., 2009). For years we have been using conventional immunohistochemistry to elucidate biochemical pathways and identify neurotransmitters in mammalian taste buds. The disadvantages of this technique are that the results are capricious and the length of the experiment after fixation and labeling takes large amounts of time, typically three days. For many antibodies, an additional antigen retrieval step is required. This, however, can be problematic, because the tissue is easily damaged during antigen retrieval and tissue preservation can be compromised. Recently, however, microwave tissue processing has begun to emerge as an efficient and, in some cases, superior technique when compared with conventional immunohistochemical preparation techniques.

Microwave irradiation was first utilized as a fixation method in the laboratory in 1970 (Mayers, 1970). Since then, microwave processing has been incorporated into many techniques, resulting in a significant decrease in the amount of time necessary to complete a procedure, better fixation, improved preservation of tissue, and enhanced immunostaining (Leong et al., 1985, 1988; Miura et al., 1988; Boon et al., 1990; Takes et al., 1989; Leong and Milios, 1990; Krug and Takes, 1991; Kok and Boon, 1990, 1994, 2003; Login and Dvorak, 1988, 1994; Leong and Sormunen, 1998; Giberson and Demaree, 2001; Giberson et al., 2003; Munoz et al., 2004; Leong and Price, 2004; Galvez et al., 2006; Ferris et al., 2009). When microwaves are applied to a solution, they cause any electrically charged molecule to rapidly rotate throughout the sample, resulting in an increase in diffusion rates. In a typical microwave processor, this would result in an increase in temperature, thereby heating the sample. Specialized microwave tissue processors allow one to control variables such as temperature, wattage, and time. In these processors, microwaves are applied to tissue samples that

Abbreviations: PLCβ2, phospholipase Cβ2; IP_3R3, inositol 1,4,5-trisphosphate receptor 3; I.P., intraperitoneal; PLP, periodate–lysine–paraformaldehyde; PFA, paraformaldehyde.

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Table 1
Primary antibodies.

Antibodies	Species	Dilution	Source	Code no.
α-Gustducin	Rabbit	1:100	Santa Cruz Biotech.	SC-395
IP_3R3	Mouse	1:100	Transduction Lab.	610313
PLCβ2	Rabbit	1:100	Santa Cruz Biotech.	SC-206
Syntaxin-1	Mouse	1:100	Sigma	S0664

Table 2
Secondary antibodies and nuclear stain.

Antibodies/stains	Species	Dilution	Source	Code no.
Dylight 649 IgG	Rabbit	1:100	Jackson	111495144
AlexaFluor 546 IgG	Mouse	1:100	Molecular Probes	A11030
Sytox	N/A	1:100	Molecular Probes	S7020

are suspended in solution. Electrically charged molecules begin to move rapidly throughout the sample; however, the temperature is controlled to prevent overheating or "cooking" the sample. The use of a vacuum in microwave tissue processing enhances tissue preservation and facilitates diffusion resulting in deeper penetration of antibodies.

There were initially many limitations to the use of microwaves in laboratories. Temperature and wattage had to be closely monitored throughout the procedure and it was difficult to provide a uniform distribution of microwave irradiation throughout the sample. With the current generation of microwave processors, these problems are greatly reduced. Temperature, wattage, and time can be controlled automatically. Although all the antibodies in this study reacted similarly to microwave irradiation, there is evidence that some antibodies react differently (Hjerpe et al., 1988; Takes et al., 1989; Munoz et al., 2004; Temel et al., 2006). The temperature, wattage, and time must be adjusted based on the nature of the antibody. Other studies have shown that controlling temperature and power output can affect results (Sanders and Gartner, 2001; Sanders, 2002; Munoz et al., 2004; Ferris et al., 2009). Using a modern laboratory microwave tissue processor, these settings can be automated and easily changed before and after an experiment. Protocols are entered and stored on the microwave's touch screen system so that the procedure can be carried out automatically with the press of a button. Variables for each protocol can be changed at any time.

In the present study we have studied the efficacy of microwave processing for immunohistochemistry on circumvallate taste buds of rodents. We have compared results from tissues using the conventional method of immunohistochemistry with those from tissues using a microwave processor to evaluate the effects of microwave-assisted immunohistochemistry on taste cells.

2. Materials and methods

2.1. Animal care

Adult Sprague-Dawley male rats (250–350 g) were used for these studies. Animals were cared for and housed in facilities approved by the Institutional Animal Care and Use Committee of the University of Denver. All animals were anesthetized with a mixture of sodium ketamine (200 mg/kg) and xylazine (70 mg/kg) (I.P.). Primary antibodies, secondary antibodies, and nuclear stain are listed in Tables 1 and 2.

2.2. Conventional immunohistochemistry for confocal microscopy

Four rats were perfused for 10 s through the left ventricle with 0.1% sodium nitrate, 0.9% sodium chloride and 100 units of sodium heparin in 100 ml of 0.1 M phosphate buffer (pH 7.3). This was followed by perfusion fixation with PLP (75 mM Lysine, 1.6% PFA, and 10 mM Sodium Periodate) in 0.1% phosphate buffer for 10 min (Weedman et al., 1996). All perfusates were warmed to 42 °C before use. After perfusion, the excised circumvallate papillae were fixed in fresh fixative for 3 h at 4 °C. The tissues were then cryoprotected with 30% sucrose in 0.1 M phosphate buffer overnight at 4 °C.

2.3. Double labeling and nuclear staining

Tissue was frozen in OCT and then sliced on a cryostat into sections 20 μm thick. Cryostat sections containing circumvallate taste buds were washed in 0.1 M PBS (pH 7.3) for 30 min, then blocked in 5% normal goat serum and 0.3% Triton X-100 in 0.1 M PBS (pH 7.3) for 2 h on ice. An additional step was added to the procedure when the primary antibody to IP_3R3 was applied: sections were incubated in 10 mM sodium citrate (pH 9) for 20 min at 80 °C for antigen retrieval, prior to blocking. The sections were incubated in a combination of two primary antibodies: either rabbit polyclonal antibody α-gustducin with mouse monoclonal antibody IP_3R3 or rabbit polyclonal antibody PLCβ2 with mouse monoclonal syntaxin-1. Both combinations were mixed with 0.1 M PBS (pH 7.3) and refrigerated overnight at 4 °C. Tissues were then rinsed in 0.1 M PBS (pH 7.3) for 30 min. The sections were treated in a combination of two secondary antibodies in 0.1 M PBS (pH 7.3): Alexa-Fluor 546 goat anti-mouse IgG and Dylight 649 goat anti-rabbit IgG. The tissues were left in this treatment for 1 h. Following this treatment, the tissues were washed in 0.1 M PBS (pH 7.3) for 30 min. For nuclear staining, the tissues were washed in 0.1 M PBS (pH 7.3) for 10 min, followed by 1 min of incubation in a mixture of Sytox nuclear stain and deionized distilled H_2O. Following this incubation, tissues were washed for 30 min in deionized distilled H_2O. The sections were then mounted onto glass slides using Fluorogel with Tris Buffer (Electron Microscopy Sciences).

All sections were processed in polystyrene 6 wells plates, with approximately 2.5 ml of the appropriate solution in each well for rinsing. During antibody incubation steps, tissue was immersed in 500 μl of solution in a 1 ml centrifuge tube.

2.4. Microwave processed immunohistochemistry for confocal microscopy

Four rats were perfused for 10 s through the left ventricle with 0.1% sodium nitrate, 0.9% sodium chloride and 100 units sodium heparin in 100 ml 0.1 M phosphate buffer (pH 7.3). This was followed by perfusion fixation with PLP (75 mM Lysine, 1.6% PFA, and 10 mM Sodium Periodate) or 4% PFA in 0.1% phosphate buffer for 10 min (Weedman et al., 1996). All perfusates were warmed to 42 °C before use. After perfusion, the excised circumvallate papillae were fixed in fresh fixative in a Ted Pella Biowave Microwave Processor inside the Pelco EM Pro Vacuum Chamber, connected to the microwave processor by the Pelco ¼″ Silicone Vacuum Hose, for 6 min. The vacuum power remained on for the entire 6 min. During this time, the microwave power was turned on at 150 W for 2 min, turned off for 2 min, and then turned back on at 150 W for 2 min. Alternating the on–off state of the microwave power helps to maintain temperature at 23 °C or below and increase the speed of antibody interactions, resulting in increased antibody penetration over a reduced time span. The tissue was then cryoprotected in 30% sucrose in phosphate buffer (pH 7.3) in the microwave processor with vacuum for 6 min. During this time, the microwave power was turned on at 150 W for 2 min, turned off for 2 min, and then turned back on at 150 W for 2 min. These steps were carried out in polystyrene 6 well plates, with approximately 0.5 ml of the appropriate solution in each well.