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Vibratory stimulation enhances thyroid epithelial cell function

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ABSTRACT

The tissues of the body are routinely subjected to various forms of mechanical vibration, the frequency, amplitude, and duration of which can contribute both positively and negatively to human health. The vocal cords, which are in close proximity to the thyroid, may also supply the thyroid with important mechanical signals that modulate hormone production via mechanical vibrations from phonation. In order to explore the possibility that vibrational stimulation from vocalization can enhance thyroid epithelial cell function, FRTL-5 rat thyroid cells were subjected to either chemical stimulation with thyroid stimulating hormone (TSH), mechanical stimulation with physiological vibrations, or a combination of the two, all in a well-characterized, torsional rheometer-bioreactor. The FRTL-5 cells responded to mechanical stimulation with significantly ($p < 0.05$) increased metabolic activity, significantly ($p < 0.05$) increased ROS production, and increased gene expression of thyroglobulin and sodium-iodide symporter compared to un-stimulated controls, and showed an equivalent or greater response than TSH only stimulated cells. Furthermore, the combination of TSH and oscillatory motion produced a greater response than mechanical or chemical stimulation alone. Taken together, these results suggest that mechanical vibrations could provide stimulatory cues that help maintain thyroid function.

1. Introduction

The tissues of the body are routinely subjected to various forms of mechanical vibration, the frequency, amplitude, and duration of which can contribute both positively and negatively to human health [1]. Small doses of vibration may promote tissue growth [2], while large doses can result in tissue damage [3]. An example of the later is hand-arm vibration syndrome (HAVS), where the operation of vibrating power tools can produce chronic and progressive dysfunction to the vascular, muscular, and neurological systems [4,5]. Several studies on HAVS have demonstrated a connection between mechanical vibrations and an adverse cellular response, such as vascular complications in response to increased reactive oxygen species (ROS) production [6–8]. In contrast, whole body vibrations have been positively associated with substantial increases in hormone production [9–11], possibly through direct mechanical stimulation or enhanced biomolecular transport.

These studies suggest that mechanical vibrations could play an important role in regulating hormone production and ROS in the endocrine system. The most common endocrine disease, hypothyroidism, affects approximately 5% of the population [12]. It is characterized by a decrease in thyroid hormone production that stems in part from a reduction in thyroid epithelial cell sensitivity to thyroid stimulating hormone (TSH). In order for these hormones to be produced, iodine must be transported into the thyroid epithelial cells through the sodium iodine symporter (NIS). Iodine must then be oxidized by thyroid peroxidase (TPO) and the ROS hydrogen peroxide (H_2O_2) and added to the tyrosine residues of thyroglobulin (TG). The iodized tyrosine residues are then cleaved to form the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4), which are critical for regulating cell metabolism, growth, and development [13].

There is some evidence that mechanical stimulation can positively influence TSH sensitivity. Several in vitro studies have demonstrated that thyroid epithelial cells are responsive to their mechanical environment, particularly in the context of altered gravity and space exploration [14,15]. For example, Meli *et al.* found that Fischer rat thyroid line-5 (FRTL-5) cells had decreased sensitivity to thyroid stimulating hormone (TSH) after exposure to a hypogravity situation [15]. In contrast, FRTL-5 exposure to a 7*g* centrifugal force increased TSH receptor (TSHR) number and responsiveness to TSH [14]. The difference in outcomes could be in part due to an increase in reactive oxygen species (ROS) production in response to an altered mechanical environment, particularly as ROS are vital to thyroid hormone

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production. Too much ROS, however, can be detrimental to thyroid health [16,17], with excess ROS implicated as the basis for the high rate of cancer found in the thyroid [18].

The vocal cords, which are in close proximity to the thyroid, may also supply the thyroid with important mechanical signals that modulate hormone and ROS production via mechanical vibrations from phonation. In order to explore the possibility that vibrational stimulation from vocalization can enhance thyroid epithelial cell function, we subjected FRTL-5 cells to physiological vibrations in a well-characterized, torsional rheometer-bioreactor [19–21] and compared their response to TSH stimulated cells.

2. Methods

2.1. Cell culture

Fischer rat thyroid line cells (FRTL-5, American Type Culture Collection, Rockville, MD, CRL 8305) derived from the normal thyroid gland of Fischer rats were maintained in a 37 °C humidified incubator supplied with 95%/5% air/CO_2. FRTL-5 cells were cultured according to the supplier's recommendations in Ham's F12K medium with 2 mM L-glutamine and adjusted to contain 1.5 g/L sodium bicarbonate. 0.5% bovine calf serum and six additional hormones required for FRTL-5 cells to proliferate and maintain thyroid function were added to the medium, as specified by the originators of this cell line [22,23]. These six hormones included 1 mU/mL thyroid stimulating hormone (TSH), 0.01 mg/mL insulin, 10 nM hydrocortisone, 0.005 mg/mL transferrin, 10 ng/mL somatostatin, and 10 ng/mL glycyl-L-histidyl-L-lysine acetate (All reagents from Sigma, St Louis, MO) [24]. Growth medium was used for cell expansion and to acclimate the cells prior to initiating the experiments. For the experiments, growth medium was modified so that it either contained 10 mU/mL TSH or no TSH as indicated below. Cells were passaged at 70% confluency and harvested at passages four through eight for the experiments.

2.2. Application of physiological vibrations with a torsional rheometer-bioreactor

With the Torsional Rheometer-Bioreactor (TRB) previously developed in our lab [19], physiological forces can be applied to adherent cells in a multi-well disc by specifying the frequency, amplitude, and duration of vibration (Fig. 1). Briefly, 96 well tissue-culture plates were cut into 57 mm diameter, multi-well discs so that the plates could be positioned in the TRB. The plates, which were previously sterilized by the manufacturer, were covered with a sterile adhesive to prevent debris from entering the wells during cutting with a jigsaw and to preserve sterility. Next, trypsin/EDTA (Life Technologies, Grand Island, NY) was used to release FRTL-5 cells from the tissue culture flasks. The cells were centrifuged at 1500 rpm for 5 min, re-suspended in complete media, and then plated at a density of 10,000 cells/well to each of the eight outer wells of the disc, each of equal radial distance from the disc center. FRTL-5 cells were also re-plated at the same density in wells of a 96-well plate to serve as controls (n=8). Cellular attachment was allowed to occur in all wells for 48 h in growth medium (i.e., with 1 mU/mL TSH) before TSH was completely removed from the growth medium for an additional 48 h of culture. TSH deprivation between 1 and 10 days is frequently employed (e.g., [25,26]) prior to beginning an experiment because it greatly increases FRTL-5 sensitivity to the reintroduction of TSH [22], and because deprivation is thought to control cell synchronization [27]. We chose a deprivation period of 48 h followed by re-exposure to 10 mU/mL TSH based on other studies that used similar parameters [28,29]. In particular, we used the study by Bjorkman *et al.* [28] as a guide, where they showed a significant increase in hydrogen peroxide production with two days of TSH deprivation followed by 10 mU/mL of TSH. Immediately following this 48 h TSH-free culture period, 10 mU/mL of TSH was added back to growth medium supplied to half of the wells in the multi-well disc (n=4) and to half of the wells in the control plate (n=4). The other half of the wells received growth medium without any TSH. The multi-well discs were then mounted onto the TRB and subjected to four conditions: (1) no oscillation and no TSH; (2) no oscillation and TSH; (3) oscillation and no TSH, (4) oscillation and TSH. The oscillatory conditions for this study were selected based on vibrational parameters related to vocalization [30]. The TRB was set up to apply inertial forces to the adherent cells in the form of oscillatory accelerations of 2 m/s^2, based on accelerations measured on the skin in front of the thyroid [31]. In addition, the experiments were conducted at an torsional frequency of 126 Hz, a frequency within the range of a typical adult male voice and corresponding to the characteristics of American speech and the daily voice usage of public school teachers [30]. Finally, the stimulation was applied constantly (i.e., a duty ratio of 1) for the full duration of the experiment (i.e., four hours) in order to produce for this pilot study what we hypothesized would be a maximum effect from mechanical stimulation. Four hours was selected in order to be consistent with prior experiments conducted with vocal fold fibroblasts [19] and because *t*-tests from a preliminary study indicated significant differences in metabolic activity (p=0.0154) between TSH deprived and TSH stimulated groups after only four hours.

2.3. Quantification of metabolic activity

Alamar Blue (alamarBlue®, Life Technologies, Grand Island, NY) was used to quantify differences in metabolic activity in response to exposure to TSH and oscillatory accelerations [32]. A 10x stock solution of Alamar Blue was diluted in calcium and magnesium free PBS to achieve a 1x final concentration. At the conclusion of each experiment, medium was removed, the wells were washed gently with PBS, and 100 μL of Alamar Blue solution was added to each well. Both the multi-well discs and controls were returned to the incubator for 1 h. The Alamar Blue solution was then transferred to a black 96-well plate and the fluorescence was measured (Ex/Em=560/590 nm) with a fluorometer (FLOUstar, BMG LABTECH Ltd., Ortenberg, Germany).

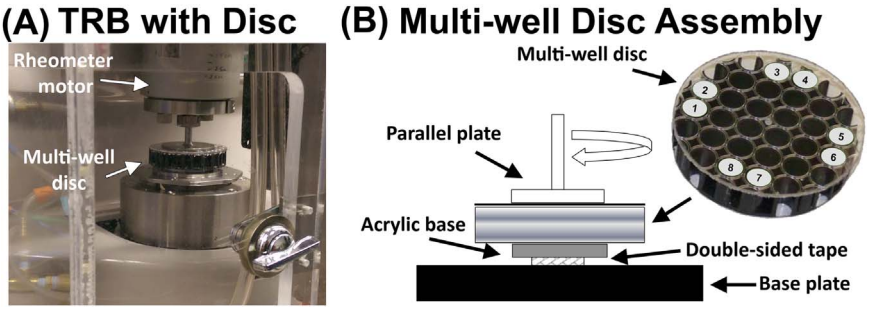

Fig. 1. Torsional Rheometer Bioreactor (A) Image of the TRB enclosed in an environmentally controlled chamber. (B) Schematic of the components of the multi-disc assembly, including the locations of the eight wells used in each study. Four of the wells were chemically stimulated with TSH in addition to receiving oscillatory mechanical signals. The other four wells received no TSH.