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Theophylline Increases the Uptake of Radioiodine by Mouse Thyroid

Diagnostic and therapeutic use of radioiodine in the management of thyroid disorders depends on the ability of thyroid cells to concentrate radioiodine, a process that is regulated by the intracellular increase in cAMP. We hypothesized that theophylline, a drug known to increase intracellular cAMP via inhibition of phosphodiesterase, could increase thyroidal radioiodine uptake. We tested this effect in vivo, using C57BL/6j mice, and in vitro, using Fisher rat thyroid (FRTL-5) cells. One mouse received 2.5 mg theophylline i.p., whereas a control mouse received only saline. Twenty-hours after theophylline, mice were injected with 10 μCi $Na^{125}I$ in 0.1 mL saline through the tail vein. Mean thyroidal ^{125}I activity was 3.3-fold higher in theophylline-treated mice than in their respective controls. Radioiodine uptake and intracellular cAMP production of FRTL-5 cells were increased by a relatively low concentration of theophylline (1 μM). Intracellular cAMP increased up to 30 min and then declined in response to 1 μM theophylline. Sera from theophylline-treated mice stimulated ^{125}I uptake and intracellular cAMP production by FRTL-5 cells. These findings show that theophylline can enhance radioiodine uptake by thyrocytes in vivo and in vitro. The in vitro effects of theophylline on both radioiodine uptake and cAMP production in a dose-dependent manner are consistent with an action mediated by phosphodiesterase inhibition.

Key Words : *Theophylline; Iodine Radioisotopes; Cyclic AMP; Thyroid Gland*

Jong Min Lee, Patrizio Caturegli*, Paul W. Ladenson†

Department of Internal Medicine, The Catholic University of Korea, Medical College, Seoul, Korea; Department of Pathology, Molecular Microbiology, and Immunology* and Department of Medicine†, The Johns Hopkins Medical Institutions, Baltimore, Maryland, USA

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Address for correspondence
Jong Min Lee, M.D.
Department of Internal Medicine, Division of Endocrinology and Metabolism, The Catholic University of Korea, Daejon St. Mary's Hospital, 520-2 Daeheung-dong, Jung-gu, Daejon 301-723, Korea
Tel : +82.42-220-9512, Fax : +82.42-255-8663
E-mail : drjmlee@catholic.ac.kr

INTRODUCTION

Diagnostic and therapeutic use of radioiodine in thyroid disorders depends on the ability of thyroid tissue to concentrate radioiodine, a thyrotropin (TSH)-stimulated process mediated by cAMP augmentation of iodine trapping, oxidation, and organification. Various methods have been tried to enhance thyroidal uptake of radioiodine, including a low iodine diet (1) and diuretics (2) to reduce body content of stable iodine and lithium to increase the retention of radioiodine in thyroid cells (3-5).

Theophylline, a methylxanthine derivative which inhibits cellular phosphodiesterases and preserves intracellular cAMP, may potentiate the effect of TSH. Thirty years ago, Ahn and Rosenberg observed that theophylline increased iodine organification (6). After this report, there were few studies which assessed the direct effect of theophylline on the thyroidal radioiodine uptake in vitro or in vivo and most of these studies were done before the 1980s. The aim of this study was to address the hypothesis that theophylline would augment radioiodine uptake in vivo by normal mouse thyroid tissue and in vitro rat thyrocytes in cultures.

MATERIALS AND METHODS

Thyroidal radioiodine uptake in mice

Ten pairs of adult female C57BL/6j mice were studied. In each pair, one mouse received 2.5 mg theophylline i.p. dissolved in 0.5 mL 0.9% sterile saline, whereas its control received only saline. Twenty-hours after theophylline, 10 μCi $Na^{125}I$ (ICN Biochemicals Inc., Cleveland, OH, U.S.A.) in 0.1 mL 0.9% sterile saline were injected through the tail vein to all mice. Four hours later, the mice were sacrificed, their sera collected, and thyroid glands dissected and counted in a gamma counter (Packard Instrument Co., Downers Grove, IL, U.S.A.). To determine background radioactivity, a piece of quadriceps muscle was dissected, weighed, counted and its radioactivity per weight subtracted from that of thyroid tissue. Radioiodine uptake was expressed as cpm/mg of thyroid tissue. All of the animals were maintained in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and all experimental procedures were approved by the institutional animal use and care committee for use of vertebrate animals in research.

Radioiodine uptake by FRTL-5 cells

The in vitro uptake of $Na^{125}I$ by rat thyroid FRTL-5 cells (Interthyr Research Foundation, Baltimore, MD, U.S.A.; ATCC CRL 8305) was measured as described by Weiss et al. (7) with slight modification. Briefly, cells were grown in 24-well plates at 37°C in 5% CO_2, for 2-3 days in Coon's modified F-12 medium containing 5% heat-treated, mycoplasma-free calf serum (Life Technologies), 1 mM non-essential amino acids (Life Technologies) supplemented with a mixture of six hormones, including 10 μg/mL insulin, 0.4 ng/mL cortisol, 5 μg/mL transferrin, 2 ng/mL glycyl-L-histidyl-L-lysine acetate, 10 ng/mL somatostatin and 10^{-10} M bovine TSH. These hormones, except cortisol (Calbiochem, La Jolla, CA, U.S.A.), were obtained from Sigma (St. Louis, MO, U.S.A.). Cells were exposed for various times to different concentrations of theophylline (Sigma), either added directly to culture medium or to serum drawn from a mouse that had been injected with theophylline. After aspirating the culture medium, cells were washed with 1 mL of buffer of following composition: Hanks' balanced salt solution (Life Technologies), which contains calcium and magnesium but does not contain sodium bicarbonate, phenol red, or BSA, with 10 mM HEPES buffer (Life Technologies), pH 7.3 (HBSS). ^{125}I uptake was initiated by adding 0.5 mL of warm buffered HBSS containing 10 μM NaI and $Na^{125}I$ (0.1 μCi/mL, to have around 100,000 cpm per well). Incubations proceeded for 45 min at 37°C water bath, and were terminated by aspirating the radioactive medium and washing with 1 mL of ice-cold HBSS twice. To determine the amount of $Na^{125}I$ associated with the cells, 1 mL of ice-cold absolute ethanol was added to each well and incubated for 3 hr at 4°C. The supernatants containing alcohol extracts were transferred into vials for gamma counting. Radioiodine uptake is expressed as cpm per optical density of DNA in each well measured in an ELISA reader (Dynatech Laboratories, Chantilly, VA, U.S.A.) after reacting the cells with diphenylamine solution (Sigma) overnight (8).

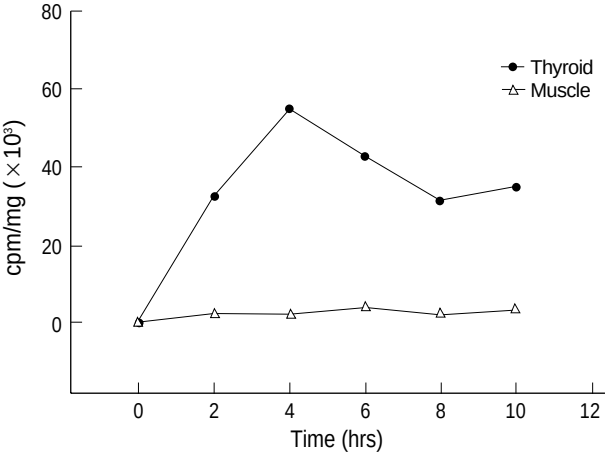

Fig. 1. Kinetics of radioiodine uptake in normal mice. Two C57BL/6j mice for each time point received 5 μCi $Na^{125}I$ in 0.1 mL saline by tail vein injection.

Measurement of FRTL-5 cell intracellular and total cAMP

Intracellular and total cAMP levels of FRTL-5 cells were measured using a commercial cAMP enzymatic immunoassay kit (Amersham Pharmacia Biotech Inc, Piscataway, NJ, U.S.A.). Cells were plated in 96-well microtiter plates, at a concentration of 1×10^6 cells/mL, and incubated overnight at 37°C in 5% CO_2. After stimulating the cells with medium containing different concentrations of theophylline or serum, a lytic solution containing dodecyltrimethylammonium bromide was either added after aspirating the culture media for subsequent intracellular cAMP quantitation or directly introduced into culture media for total cAMP measurement. Lysis of the cells was performed for 10 min by shaking the plate on a microtiter plate shaker. Standard solutions and samples from each well pipetted into microtiter plates coated with donkey anti-rabbit IgG. Optical density in each plate was determined reader at 450 nm, following the procedure recommended by manufacturer.

Statistical analysis

Student's t test and one-way ANOVA were used. Differences were considered statistically significant at $p<0.05$.

RESULTS

Radioiodine uptake study in normal mice

The kinetics of radioiodine uptake in normal mice thyroid tissue and muscle after tail vein injection was first studied. Peak thyroidal uptake was observed at 4 hr after injection (Fig. 1), which led us to measure radioiodine uptake at this time point in future experiments. Within each pair of mice, net thyroidal ^{125}I activity was greater in all ten of the theophylline-treated mice, 440,451±383,362 vs. 196,037±235,435

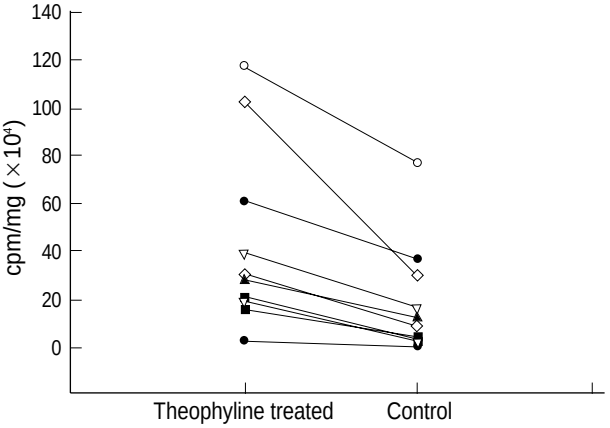

Fig. 2. In vivo thyroidal radioiodine uptake.

cpm/mg (mean±SD, $p<0.01$ by Student's paired t test), even though there was considerable variation in the degree of uptake among the pairs. Overall, thyroidal ^{125}I activity was 3.3-fold higher in theophylline-treated mice than in their respective controls (range 1.5- to 6.7-fold increases) (Fig. 2). No mice died before sacrifice or manifested behavior suggesting theophylline toxicity.

Radioiodine uptake of FRTL-5 cells in response to theophylline

When FRTL-5 cells were incubated with theophylline in different concentrations for more than 30 min up to 20 hr, the same exposure time employed for in vivo studies, intracellular ^{125}I activity was not significantly different with any theophylline concentration from that of cells incubated with medium alone (data not shown). FRTL-5 cells were then exposed to different concentrations of theophylline (1, 10, 100 and 200 μM) for 30 min. Only 1 μM theophylline significantly increased intracellular ^{125}I activity compared to cells incubated with medium only (43,598±3,011 vs. 31,804±3,963 cpm/DNA [O.D], means of triplicate±SD, $p=0.015$) (Fig. 3). However, no effect was observed with theophylline concentrations that were comparable to therapeutic serum concentrations for bronchial asthma (100 μM) and higher (200 μM).

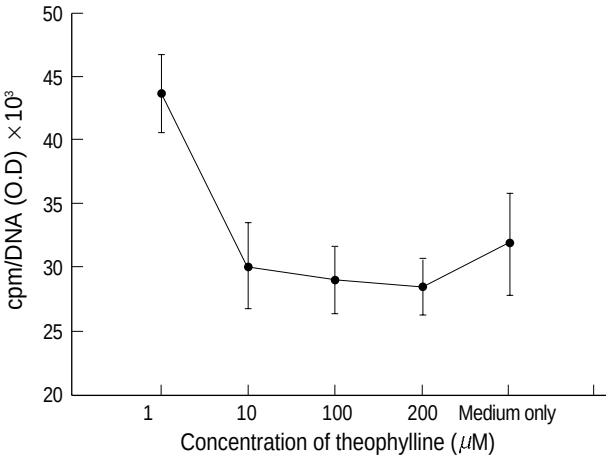

Fig. 3. Radioiodine activity in FRTL-5 cells in response to theophylline.

Intracellular and total cAMP response of FRTL-5 cells to theophylline

We investigated the time course of the intracellular cAMP concentrations of FRTL-5 cells in responses to 1 μM theophylline. Intracellular cAMP increased up to 30 min and then declined significantly (159±28 at basal, 688±104 at 30 min, 306±40 pmol/well at 120 min, means of triplicate± SD, $p=0.001$) (Fig. 4A). FRTL-5 cells were then exposed to various concentrations of theophylline for 30 min. Intracellular cAMP concentration of the cells incubated with 1 μM theophylline was higher than that of cells incubated with medium only (528 vs. 109 pmol/well, mean of duplicates). Although no significant increase in intracellular cAMP was observed at higher theophylline concentrations (10, 100 and 200 μM), total cAMP (cellular+medium cAMP) was increased in a dose-dependent manner throughout the studied theophylline dose range (Fig. 4B).

In vitro radioiodine uptake and cAMP responses to mouse sera

FRTL-5 cells were incubated for 30 min with control mouse serum or serum taken 20 h after the mouse had received theophylline 2.5 mg i.p. Theophylline-treated mouse serum significantly increased radioiodine uptake by FRTL-5 cells at dilutions 1:200 and 1:100 compared to control mouse serum (46,531±4,577 vs. 36,916±3,674 cpm/DNA [O.D] at 1:200, 48,529±4,455 vs. 35,982±4,154 cpm/DNA [O.D]

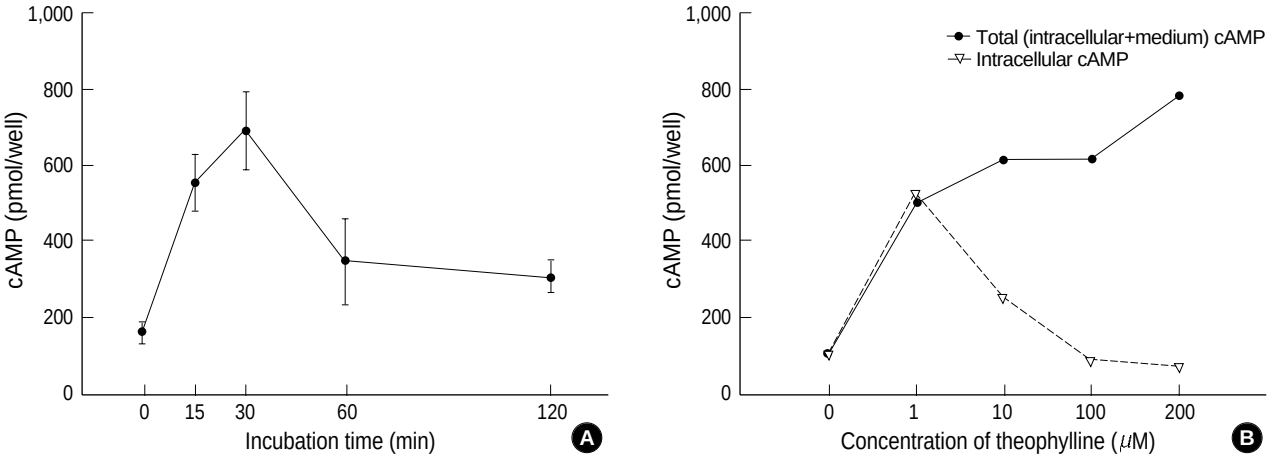

Fig. 4. cAMP concentration in FRTL-5 cells in response to theophylline. Cells were cultured in triplicate (A) or in duplicate (B) with cell concentration of 10^6 cells/mL in 96-well culture plates. (A) FRTL-5 cells were incubated with 1 μM theophylline for various time periods. (B) Cells were stimulated with various concentrations of theophylline for 30 min.

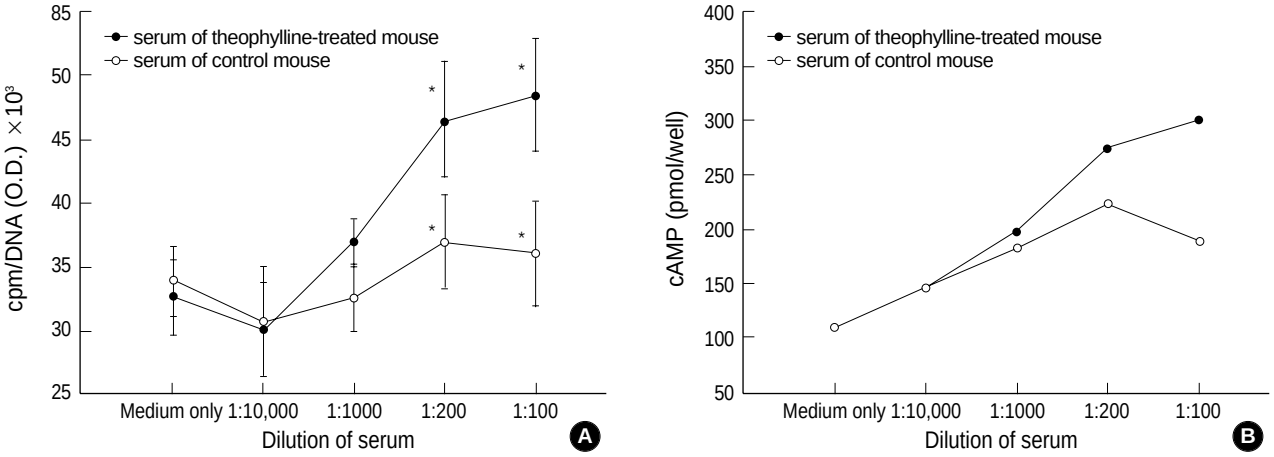

Fig. 5. Radioiodine uptake and intracellular cAMP production by FRTL-5 cells in response to serum. FRTL-5 cells were cultured in triplicate in 24-well culture plates to measure radioiodine uptake (A), and in duplicate in 96-well culture plates for cAMP measurement (B).

at 1:100, means of triplicate ± SD, $p<0.05$) (Fig. 5A). Serum from the theophylline-treated mouse also showed a greater intracellular concentration of cAMP within FRTL-5 cells compared to control mouse serum (303 vs. 191 pmol/well at 1:100 dilution, mean of duplicates) (Fig. 5B).

DISCUSSION

The transport of iodide, the first step in thyroid hormone biosynthesis, is a specialized process performed most efficiently by thyrocytes. The ability of TSH to stimulate iodide accumulation in the thyroid was first established in 1960 (9). In rats, injection of TSH caused 50-100% increase in thyroid iodide accumulation after 8 hr, as measured by the thyroid-to-serum radioiodide gradient. The maximum increase was detected 24-48 hr after injection. Similar experiments conducted in the 1970's in isolated bovine thyroid cells also showed 50-100% stimulation of accumulation 6 hr after addition of TSH in vitro (10, 11). This effect of TSH was reproduced by dibutyryl cAMP and by agents that increased intracellular levels of cAMP, such as the diterpene forskolin, further suggesting that the effect of TSH on the iodide transport system is mediated by cAMP (6, 7, 11, 12). In addition to iodide uptake, it is generally accepted that TSH stimulation of other steps in thyroid hormone biosynthesis, including thyroglobulin biosynthesis and thyroid hormone release, requires an increase in adenylate cyclase and intracellular cAMP content (13, 14).

Theophylline inhibits the activity of phosphodiesterase, which catalyzes degradation of cAMP to AMP. Consequently, it can increase the intracellular cAMP level and augment cAMP-mediated intracellular responses in thyrocytes (15-18). Bastomsky and McKenzie (15, 16) found that theophylline had a stimulatory effect on the thyroid and potentiated the effects of TSH. Ahn and Rosenberg (6) observed prompt stimulation of thyroid hormone production by cAMP and TSH injected into rats, and similar effects of theophylline. Wolf and Varrone (19) reported that simultaneous administration of theophylline and propylthiouracil potentiated the goitrogenic effect of the latter without increasing the plasma TSH level. Kapitola et al. (20) found an increase in radioiodine uptake by thyroid in rats and mice after 4 i.p. injections of 1 or 10 mg theophylline, even when endogenous secretion of TSH was blocked by thyroxine. Gafni and Gross (21) reported a large dose of TSH combined with theophylline doubled the level of the thyroidal cAMP compared with TSH alone in normal mice.

We report in vivo and in vitro studies that more fully defined the time- and dose-dependent effects of theophylline on cAMP production and radioiodine uptake. We have investigated effects of theophylline on both murine thyroidal radioiodine uptake and on intracellular accumulation of radioiodide in cultured FRTL-5 cells in which simultaneous cAMP determinations could be made. FRTL-5 cells are known to express many characteristics of thyroid follicular cells in vivo, except that they do not form the follicular architecture of intact thyroid tissue. Specifically, FRTL-5 cells concentrate iodide approximately 30-fold, mimicking the iodide accumulation observed in thyroid tissue slice and primary cell culture systems (7).

Both the intracellular cAMP concentration and radioiodine uptake of FRTL-5 cells increased 30 min after exposure to either 1 μM theophylline directly or in serum from a theophylline-treated mouse. The rapid onset of this in vitro theophylline effect contrasts to that of TSH (9-11) and may be attributable to its more proximate action to increase the intracellular cAMP concentration. This theophylline effect was reversed when FRTL-5 cells were exposed to concentrations of theophylline greater than 1 μM or incubated for more than 30 min. A direct cytotoxic effect of theophylline or radioiodine on FRTL-5 cells seems unlikely since the cellular DNA determined for each well was not significantly different after incubation with theophylline or radioiodine for various time and concentrations. Low theophylline concentrations may have

maximally inhibited phosphodiesterase activity, and higher levels may simply result in the egress of cAMP from cells into the medium. Previous studies employing methylxanthines to study TSH-mediated changes in cAMP have shown a similar phenomenon: the initial rise in intracellular cAMP in thyrocytes subsequently declined after 30 min, principally due to the release of nucleotide into media (22-25).

In contrast to these in vitro results, radioiodine uptake by the intact murine thyroid was higher even 20 hr after exposure to theophylline. This finding suggests that intact thyroid cells may be able to sustain augmented cAMP levels and not become desensitized despite sustained phsophodiesterase inhibition. It is also possible that the membrane of thyroid cells in vivo may remain relatively impermeable to cAMP under physiologic conditions.

There are few reports of long-term effects of theophylline on the thyroid function. Schreiber et al. (26) reported that stimulatory effect of theophylline on the radioiodine uptake by rat thyroid is only of transitory nature and does not persist more than 4 weeks. In humans, short-term administration (less than 4 weeks) of theophylline increased serum free thyroxine and free triiodothyronine concentrations, but there was no sustained effect with long-term therapy (27). Other investigators have reported that the short-term increase in serum thyroxine concentration induced by theophylline was correlated with the rise in plasma cAMP (28).

Our findings clearly support the hypothesis that theophylline can enhance cAMP-mediated radioiodine uptake by thyrocytes in vivo and in vitro. The effects of theophylline in our studies both cAMP production and radioiodine uptake were time- and dose-dependent, consistent with an action mediated by phosphodiesterase inhibition. Augmenting thyroidal radioiodine uptake by theophylline could enhance radioisotopic approaches to diagnose and treat thyroid disorders. In this regard, it is interesting that certain well-differentiated thyroid cancers have been observed to have higher phosphodiesterase activity (29). However, the efficiency of radioiodine for treatment of thyroid cancer and hyperthyroidism, in particular, depends not only on the fractional thyroidal uptake of radioiodine but also its residence time in the gland (3-5). If theophylline accelerates the subsequent steps in thyroid hormone production that lead to the egress of organified radioiodine from the gland, then the net cellular radiation dose may not prove to be increased. Further in vivo studies will be required to address this issue.

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