In Vitro Responses of the Epidermis to Triiodothyronine

PETER J. A. HOLT, M.B., M.R.C.P.

Department of Dermatology, University of Oklahoma, Oklahoma City, Oklahoma, U.S.A.

The effect of exogenous triiodothyronine on replication and anabolic activity in human epidermis has been studied in vitro. Measurements of epidermal replication, as estimated by a labeling index technique and by thymidine incorporation into skin sheets, were increased (p < 0.05) from the control value by exogenous triiodothyronine at twice its physiological unbound concentration. Higher concentrations of triiodothyronine did not alter these elevated values significantly. Epidermal anabolism, measured by the rates of incorporation of tritiated proline and histidine, and by histidine uptake assessed autoradiographically, responded similarly with increased values (p < 0.05) compared to controls in specimens incubated with twice physiological concentrations of the hormone. Further increases in the hormone concentration failed to change these measurements significantly.

These observations indicate that the epidermis does respond to exogenous triiodothyronine in vitro, and support the concept that there are receptors for triiodothyronine associated with epidermal cells.

Changes occur in the epidermis in patients with thyroid disease [1]. Epidermal anabolism and replicative activity are increased in thyrotoxicosis and are reduced in hypothyroidism. These changes correlate significantly with serum triiodothyronine [T3] levels, but not with serum thyroxine iodine [2].

The aim of this study was to determine whether epidermal cell replication and anabolism could be affected by exogenous triiodothyronine in vitro, and to study the dose effect relationship of triiodothyronine on these epidermal measurements.

MATERIALS AND METHODS

Skin sheets from the lateral aspect of the thigh were obtained from healthy male volunteers (age-range 23–29, mean 25 yr) using a Castroviejo keratome set at 0.4 mm.

ANABOLIC ACTIVITY

Anabolic activity of the epidermis was evaluated by measuring the uptake of tritiated amino acids into skin sheets by scintillation counting and autoradiography.

I) Scintillation Method

A method was employed similar to that of Marks, Fukui and Halprin [3]. The rates of incorporation of tritiated proline (specific activity 35 Ci/mmol) and histidine (specific activity 42 Ci/mmol) into pieces of skin approximately 50 mm² in area obtained from the thigh with the keratome were measured. Five separate experiments were performed with both amino acids using skin from different donors. In each experiment skin samples from 1 donor only were used. The skin samples were incubated in Eagle’s minimal essential medium with the epidermis uppermost. Triiodothyronine, B.P. (Glaxo Laboratories, UK) was added to the medium at 1X, 2X, 4X and 8X the physiological (unbound) serum level (0.5–0.8 ng/100 ml [4]). This preparation contains not less than 95% L triiodothyronine. Aqueous solutions from the same batch were used throughout the study. The skin was then incubated for totals of 4, 8, 12 and 24 hr at 37°C in an atmosphere of 95% air and 5% CO2. Incubations without exogenous T3 served as controls. Four hours before the end of each incubation time the tritiated precursors were added to the medium in a concentration of 1 μCi/ml medium. After incubation the skin was washed and measured. The surface area of each skin sample was measured by placing the tissue on a glass cover-slip over millimeter graph paper. The tissue was homogenized and extracted with perchloric acid. The residue was solubilized with hyamine hydroxide and the incorporated radioactivity was measured in a scintillation counter. The results were expressed as corrected counts per minute (cpm)/mm² of skin/hr incubation (proline or histidine units).

II) Autoradiography

The uptake of histidine into epidermal cells was assessed autoradiographically using tritiated histidine (specific activity 42 Ci/mmol) (see below). Three experiments were performed in duplicate, each one using skin from a different donor. The number of silver grains appearing in the basal, spinous and granular layers were counted using a calibrated eyepiece and expressed as the number of grains per 100 μ² area. Measurements at each of these levels were taken at 50 random points along the epidermis.

REPLICATIVE ACTIVITY

Replicative activity of the epidermis was assessed by measuring the uptake of tritiated thymidine by autoradiography and scintillation counting.

I) Autoradiography

A similar procedure to that described under incorporation studies (see above) was followed to evaluate replicative activity. Four experiments were performed in duplicate and in each experiment skin from a different donor was used. Four hours before the end of each incubation time tritiated thymidine (specific activity 2 Ci/mmol) was added to each dish at a concentration of 1 μCi/ml medium. At the termination of each experiment the skin pieces were washed and then fixed in neutral buffered formalin. Autoradiographs were prepared using a dipping method [5] and the number of basal and suprabasal cells which became labeled were expressed as a percentage of the total number of basal cells (labeling index, LI). Cells were considered labeled if they contained 6 or more grains per nucleus.

II) Scintillation Method

The rates of incorporation of tritiated thymidine into skin sheets were estimated (see above). Five separate experiments were performed and skin samples from a different donor were
used in each experiment. Results were expressed as corrected counts per min/mm² of skin/hr incubation (thymidine units).

Quantitation of the dermal component of the epidermis. The dermal component of the keratome biopsies was quantitated by direct counting of the dermal cells in a given field, and expressing the result as the ratio of the total number of cells in the epidermis/total number of cells in the dermis, per high power field. Measurements at 10 random points on 12 different keratome biopsies were taken. The ratio was 12.7 ± 3.1:1 (± SD).

RESULTS

The results of the experiments are shown in Fig 1 and 2. Statistical evaluation was by Student’s t-test.

1. Replicative Activity (Fig 1)

The epidermal labeling index was increased (p < 0.05) by 19–33% of the control values in all specimens incubated with a twice physiological concentration (1.6 ng/100 ml) of T₃. Incubation with higher concentrations of T₃ did not further increase the labeling index significantly.

Thymidine incorporation was increased with T₃ at the physiological unbound concentration. Incubation with twice this concentration increased incorporation (p < 0.05) in the 4 hr and 8 hr specimens by 280% and 72% of the control values respectively. Higher concentrations of T₃ did not increase thymidine incorporation significantly. Values obtained with an 8 times physiological concentration were less than the peak values, but the differences were not significant at or below the 5% level.

2. Anabolism (Fig 2)

Proline incorporation increased (p < 0.05) by 31–61% of the control values in specimens incubated with a twice physiological concentration of T₃ for 4, 8 and 12 hr. Incubation with higher concentrations of T₃ did not alter these elevated values significantly.

Histidine incorporation was increased by exogenous T₃ at its physiological concentration and incubation with twice this concentration increased incorporation (p < 0.05) by 55% and 51% of the control values in the 4 hr and 8 hr specimens respectively. Higher concentration did not alter these elevated values significantly.

Histidine uptake estimated autoradiographically showed a similar trend. Incubation with twice physiological levels of T₃ increased (p < 0.05) the grain count in specimens incubated for 4, 8 and 12 hr by 25%, 41% and 53% of the control values respectively. A further twofold increase of T₃ concentration did not increase the histidine count significantly.

Uptake of the tritiated precursor compounds was generally much lower in skin specimens incubated with T₃ for 12 and 24 hr. This was seen particularly with thymidine incorporation which decreased with time with a half-life value of approximately 4 hr.

---

**Fig 1.** Epidermal replication estimated autoradiographically by a labeling index technique and by measurement of the incorporation of tritiated thymidine into skin sheets expressed as corrected counts per min/mm² tissue/hr incubation (thymidine units). Results of 4 experiments (labeling index method) and 5 experiments (thymidine incorporation). Mean ± SE of mean.

**Fig 2.** Epidermal anabolism estimated by measurement of the incorporation of tritiated proline and histidine into skin sheets and expressed as corrected counts per min/mm² tissue/hr incubation (proline or histidine units). Histidine uptake by the epidermis was also measured autoradiographically and expressed as the number of histidine grains per 100/µ² area of viable epidermis. Results of 5 experiments (proline and histidine incorporation) and 3 experiments (histidine autoradiography). Mean ± SE of mean.
DISCUSSION

It has been shown that epidermal replication and anabolic activity are significantly greater in thyrotoxicosis than in hypothyroidism [1]. These changes correlate with serum triiodothyronine concentration but not serum thyroxine iodine [2], and suggest that the receptors for thyroid hormone in human skin are specific for triiodothyronine. The observed changes in these measurements now seen in vitro reinforces this finding that the epidermis is greatly influenced by the peripheral actions of triiodothyronine.

The results indicate that epidermal proliferation is affected by triiodothyronine. The use of LI is commonly held to reflect the proliferative status of the epidermis, although this is not strictly correct as the LI will also record changes in the length of the DNA synthesis period [6]. However, in those instances in which the length of the S phase has been measured, its duration has not differed so greatly as to preclude the LI as a useful measure of epidermopoesis. Furthermore in this study, the labeling index correlated well with the rate of thymidine phosphorylation in the epidermis as a measure of epidermal cell proliferation. However inhibition of thymidine transport and phosphorylation encountered in several systems may negate the latter method as an entirely accurate measure of cell proliferation [7].

Proline and histidine incorporation were affected by exogenous triiodothyronine. The treated precursors proline and histidine are incorporated into epidermal proteins which are continuously formed in maturing epidermis and then destroyed or incorporated into proteins which are stable and appear in surface scale. Lack of precise information concerning precursor pool size prohibits deduction from the altered rates of incorporation that there are altered rates of synthesis of macromolecules. However, histidine uptake measured autoradiographically was similar to the incorporation studies. Methods of tissue preparation used for autoradiography have been shown to exclude the free amino acids administered, and the labeling observed is considered to indicate sites of synthesized protein [8].

The incorporation of precursors in these specimens was mostly epidermal and not dermal. The results of the cell counting experiment indicate that the dermal component of these specimens was comprised of comparatively hypocellular material. Autoradiographs of skin incubated with triiodothydine and histidine failed to show any dermal incorporation, so one may conclude that the incorporation of these compounds was epidermal. Autoradiographs with triiodothydine were not performed in this study. However, in view of the hypocellular dermis in these specimens it is likely that the bulk of proline incorporation is in fact epidermal too.

Nuclear binding sites for T3 [9] probably play an important role in the initiation of thyroid hormone action. Their concentration and binding capacity are greater in tissues considered to be responsive to thyroid hormone [10] and saturation of nuclear receptors with T3 in rats has been shown to induce maximal tissue response to the hormone [11].

Tata and Widnell [12] have indicated that the effect of triiodothyronine on cells is mediated by the formation of new RNA which eventuates in new protein synthesis. In this study, measurements of epidermal replication and anabolism were increased significantly from the control values by exogenous T3 at twice its physiological unbound level. It is possible therefore that there are receptors for T3 associated with epidermal cells which represent the T3 "initiation of reactions" expressed by the hormone. Further increases in the concentration of T3 failed to increase these measurements significantly. This definable maximal tissue response seen with higher concentrations of T3 suggests that the hormone receptors may be of restricted concentration and/or capacity.

Skin incubated for 12 and 24 hr generally responded poorly to exogenous T3. This was unexpected, in view of the latency of maximal response to the hormone demonstrated in rats when evaluated as amino acid incorporation into mitochondrial and microsomal protein [12,13]. However in the latter studies the response of a cell free system to near physiological concentrations of triiodothyronine was assessed, in contrast to the response of organ cultures to supraphysiological concentrations of hormone which was measured in this study. The decline in responses with longer incubation was most likely the result of decreased viability of the epidermis. Microscopic examination of the epidermis in these specimens revealed foci of vacuolation and cell death in the upper spinous layer. Minimal essential medium was used throughout this investigation because enrichment with serum would have resulted in an undetermined amount of T3 binding. A further contributory fact could have been the direct toxic effect of supraphysiological concentrations of T3 on the epidermal cells [14].

I wish to thank J. H. Anglin, Jr., Department of Biochemistry for his help during this study, and the Wellcome Trust for a Research Travel Grant.

REFERENCES


Announcement

The European Society for Dermatological Research Symposium on Vasculitis will be held at the Department of Dermatology, University of Innsbruck, February 16 and 17, 1979. This is the 4th of a series of clinically oriented symposia of the ESDDR. The session will consist of papers of invited speakers, discussion periods and free communications. Anyone wishing to contribute or to attend should contact Professor K. Wolff, Dept. of Dermatology, University of Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria.