Effect of amiodarone on L-triiodothyronine stimulation of $[^3\text{H}]$ thymidine incorporation into GH$_3$ cells

I.D. Goldfine*,**,***, B. Maddux*, and K.A. Woeber*,**
*Cell Biology Laboratory and Department of Medicine, Mount Zion Hospital and Medical Center, P.O.Box 7921, San Francisco, CA 94120, USA, **Department of Medicine, and ***Department of Physiology, University of California, San Francisco, CA 94143, USA

ABSTRACT. The antiarrhythmic agent amiodarone was found to inhibit the stimulatory effects of L-triiodothyronine on $[^3\text{H}]$ thymidine incorporation into GH$_3$ rat pituitary tumor cells. This inhibitory effect of amiodarone was detected at concentrations as low as 0.5 $\mu$M; at 2 $\mu$M greater than 50% of the stimulatory effect of L-triiodothyronine was inhibited. The effect of amiodarone was present at all concentrations of L-triiodothyronine tested (50 pM to 10 nM), suggesting that amiodarone acted as a non-competitive antagonist. These studies raise the possibility, therefore, that the effect of amiodarone on thyroid hormone metabolism may be mediated in part by an inhibition of thyroid hormone action at the cellular level.

INTRODUCTION
Amiodarone (2 butyl-3-[3,5 diodo-4 $\beta$-diethylamino- theoxybenzoyl]- benzofuran) is a potent antianginal and antiarrhythmic drug (1-3). Since amiodarone contains 2 iodine atoms and bears certain structural similarities to thyroxine, investigations have been carried out to determine whether the effects of the drug are due to interference with thyroid hormone metabolism (4). It has recently been reported that therapeutic doses of amiodarone inhibit the conversion of the less active thyroid hormone, thyroxine, to its more potent analog, L-triiodothyronine (4). However it has not been determined whether the drug also inhibits thyroid hormone action at the cellular level.

A major advance in the study of thyroid hormones has been the development of cells in tissue culture that are responsive to physiological concentrations of thyroid hormones (5). The cultured rat pituitary tumor cell (GH cell) has several biological functions that are regulated by thyroid hormones (5). In particular, thyroid hormones stimulate cell growth, allowing for a simple quantitation of biological responses. Accordingly, in the present study, we investigated whether amiodarone antagonizes the effect of L-triiodothyronine on the stimulation of $[^3\text{H}]$ thymidine incorporation into these cells.

MATERIALS AND METHODS
GH$_3$ rat pituitary tumor cells were obtained from the Cell Culture Facility, University of California, San Francisco. Cells (approximately $10^6$) were grown in 8 cm$^2$ plastic plates (Costar) with Dulbecco’s modified Eagle’s medium (DME-H21) that was obtained from the Cell Culture Facility, University of California, San Francisco; this medium was enriched with 100 U/ml penicillin, 50 $\mu$g/ml streptomycin, 2.5 $\mu$g/ml amphotericin B, and 10% fetal calf serum. Incubations were carried out at 37 C in a humidified incubator with 95% air/5% CO$_2$.

At the beginning of the experiment, cells were washed once with serum-free DME-H21 medium and then incubated with additional serum-free medium for 3 h to deplete the cells of any endogenous thyroid hormones. The serum-free medium was then removed and DME-H21 medium containing antibiotics and 10% hypothyroid calf serum (Rockland Farms, PA) was added. Next, L-triiodothyronine and amiodarone were added and the incubation was continued for 48 h. Then, 1 $\mu$Ci of $[^3\text{H}]$ thymidine (50 Ci/mMole, Amersham Corporation, Arlington Heights, IL) was added; after 30 min, the uptake of $[^3\text{H}]$ thymidine was stopped by rapidly removing the medium and washing the cells thrice with 154 mM sodium chloride at 4 C. One ml of 0.1% of sodium dodecylsulfate was added to each plate and the incubation continued for 1 h at 37 C. The cell lysates were collected, sonicated for 10 s and trichloroacetic acid (TCA) added to achieve a 10% solution. The lysates were then incubated at 4 C for 1 h and centrifuged at 1000 X g for 5 min. The pellets were solubilized in 0.1N Na0H and the radioactivity in the TCA pellets was determined in a liquid scintillation counter. Since $>$95% of the total cellular radioactivity in either the absence or presence of L-triiodothyronine and amiodarone was subsequently found to be precipitable in TCA, the TCA precipitation step was eliminated in later experiments.
Protein and glucose were measured by standard techniques (6, 7). L-triiodothyronine was purchased from Sigma Chemical Co. (St. Louis, MO) and amiodarone was a gift of Labaz N.V., Bruxelles, Belgium.

RESULTS

Stimulation of \[^{3}H\] thymidine incorporation into \(GH_3\) cells by L-triiodothyronine

After a 48 h preincubation with 1 nM L-triiodothyronine, the rate of thymidine incorporation into TCA precipitable material was doubled. Under the incubation conditions employed, this uptake was linear for up to 60 min (Fig. 1a). In subsequent experiments, therefore, a 30 min pulse was employed. The effect of L-triiodothyronine was detectable at 50 pM, one-half maximal at approximately 100 pM and maximal at 1 nM (Fig. 1b). This dose response curve for L-triiodothyronine stimulation of \[^{3}H\] thymidine incorporation was quite similar to the dose response curves for stimulation of both protein content and glucose utilization.

The effect of amiodarone on L-triiodothyronine stimulation of \[^{3}H\] thymidine incorporation

When 0.5 \(\mu\)M amiodarone was added just prior to the addition of hormone, an inhibition of the effect of L-triiodothyronine on \[^{3}H\] thymidine incorporation was detected (Fig. 2a). At 2 \(\mu\)M, approximately one-half of the L-triiodothyronine effect was inhibited. At concentrations greater than 5 \(\mu\)M, cellular toxicity (as evidenced by the presence of dead cells) was observed and there was a fall in control \[^{3}H\] thymidine incorporation.

The effect of amiodarone on the dose response of \(GH_3\) cells to L-triiodothyronine was then investigated. Over all of the concentrations of L-triiodothyronine tested, amiodarone inhibited L-triiodothyronine stimulation of \[^{3}H\] thymidine incorporation. Even at the highest dose of L-triiodothyronine tested, 10 nM, there was still a greater than 50% depression of stimulated \[^{3}H\] thymidine incorporation. Other data (not shown) indicated that amiodarone also inhibited L-triiodothyronine stimulation of both glucose consumption and protein synthesis.

Since 2 \(\mu\)M amiodarone did not alter either cell viability or control \[^{3}H\] thymidine incorporation, these observations indicated that amiodarone was not acting to