Effects of Two Thyroxine Analogs, Diiodothyropropionic Acid and Triiodothyroacetic Acid, on T-1 Kidney Cell Line[1] (35628)

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The T-1 human kidney cell line, first cultured by Veen, Bots, and Mes (1), exhibits great sensitivity and specificity for the iodinated thyronines, L-thyroxine (T_4) and 3,5,3′-L-triiodothyronine (T_3), which are elaborated by the thyroid gland. Thus, the introduction of these hormones into the growth medium affect the morphology, replication, colony-forming ability, protein synthesis, and nucleic acid metabolism of these cells (2, 3). To explore this specificity further, we have investigated the proliferation and colony-forming ability of the cell line while exposed to two active thyroxine analogs, 3,5-diiodothyropropionic acid (DTP) and 3,5,3′-triiodothyroacetic acid (TTA). Our findings using these compounds largely parallel reported observations made with other systems, extending the utility of the T-1 cells as an *in vitro* model.

Methods. Cell culture and determinations of the mean doubling time (T_d) and plating efficiency (PE) were carried out as described earlier (2), except that the growth medium contained 5% fetal bovine serum. Each experiment involved concurrent determinations on aliquot T-1 populations grown in the presence of (a) the analog, (b) a reference T_4 concentration (1.78×10^{-6} *M*), and (c) neither, as control.

For T_d: Dispersed log-phase cells (3×10^4) were seeded along with either analog (1.78×10^{-7}–1.78×10^{-5} *M*), into 100-mm diameter plastic Petri dishes; the multiplicity (4), the average number of cells per colony, at various intervals thereafter was obtained by counting 200 colonies utilizing phase-contrast microscopy; and T_d was derived from graphs of log multiplicity plotted against time after seeding. Control values of T_d for T-1 cells are about 30 hr; treatment with the reference T_4 titer (seven determinations) effects a mean relative diminution of 11.0% (range, 4.5–18.7%).

For PE: About 150 single cells were deposited with an analog (1.78×10^{-8}–1.78×10^{-5} *M*) into five replicate 60-mm diameter plastic Petri dishes; after incubation for 2 weeks and methylene blue staining, the number of visible colonies was scored relative to the quantity of cells introduced. T-1 cells have a mean PE of 58.5 ± 4.8 (SD)%, which is elevated by the reference T_4 concentration to 67.9 ± 5.6%, a relative rise of 16.1% (five experiments).

Results and Discussion. As expressed by T_d and PE, the influence DTP exerts on cellular proliferation and colony formation of the T-1 cells increases with its concentration. Representative results of T_d determinations as a function of titer of this analog are given in Table I. Thus, 1.78×10^{-6} *M* DTP (four experiments) achieves a mean relative reduction in T_d of 11.5% (range, 4.7–20.0%); a 10-fold greater concentration (three experiments) yields an average shortening of 16.7% (range, 8.7–22.8%). Summarizing five independent PE determinations, including the results of a typical experiment cited in Table I, 1.78×10^{-8} *M* DTP does not affect PE. Exposing the cultured cells to 1.78×10^{-6} *M* DTP results in a relative increase of 4.7% (range,—2.3–11.3%); a 10-fold higher concentration, the maximum studied, produces a rise of 11.8% (range, 10.0–18.0%). From

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TABLE I. Modification of Mean Doubling Time (T_d) and Plating Efficiency (PE) of T-1 Cells Produced by Diiodothyropropionic Acid (DTP), Triiodothyroacetic Acid (TTA), and Thyroxine (T_4).

Concentration (M)	Effect of DTP		Effect of TTA	
	T_d[a] (hr)	PE[b] (%)	T_d (hr)	PE (%)
1.78×10^{-8}	—	57.4 ± 3.3	—	80.3 ± 2.6
1.78×10^{-7}	22.0	58.0 ± 2.2	24.0	89.1 ± 2.5
1.78×10^{-6}	20.0	64.9 ± 5.3	25.8	84.2 ± 2.0
1.78×10^{-5}	19.3	65.0 ± 2.3	42.0	71.7 ± 1.7
Controls				
T_4 ($1.78 \times 10^{-6} M$)	20.5	63.1 ± 3.2	28.0	84.5 ± 2.9
No hormone	25.0	57.5 ± 4.9	30.3	79.5 ± 2.9

[a] T_d determinations are derived from semilog plots of multiplicity versus time from seeding; each point graphed is the mean multiplicity obtained by counting 200 colonies on duplicate dishes.

[b] PE entries are the means and standard deviations of five replicate dishes, scored for visible colonies 2 weeks after seeding and staining with methylene blue.

these experiments, it is concluded that DTP has a potency about that of T_4 in diminishing T_d, but only 0.1 its ability to augment PE.

The more marked effects on the T-1 cells elicited by TTA are indicated by Table I, which gives the results of one of three determinations made of T_d and PE. This analog provokes a biphasic response similar to that induced by T_4 and T_3 in many systems (5), including the T-1 line. While exposure to 1.78×10^{-7} M TTA, the optimal titer, causes a relative average decrease in T_d of 13.1% (range, 8.3–16.4%), the presence of 1.78×10^{-5} M actually inhibits proliferation, the mean relative increase of T_d being 12.8% (range, 4.2–38.8%). PE determinations indicate than an average relative rise of only 1.2% (range, —2.0–5.6%) occurs with 1.78×10^{-5} M and of 4.4% (range, 1.0–9.4%) with 1.78×10^{-8} M. The peak enhancement of PE, a mean relative increase of 16.1% (range, 6.3–30.2%), likewise occurs with 1.78×10^{-7} M TTA. Thus, TTA on a molar basis is at least 10 times more potent than T_4 in influencing T-1 cells.

The results of these *in vitro* investigations with DTP and TTA are qualitatively consonant with published observations using other systems. Precise comparisons are not feasible since the response to many of the thyroxine analogs varies with the end-point and model chosen. Thus, DTP has been found as active as T_4, on a molar basis, in stimulating the metamorphosis of the *Bufo* species (6), but only 1.9% as effective in elevating the metabolism of the thyroidectomized rat (7). Likewise TTA is 19–120 times more active than T_4 in stimulating the metamorphosis of the tadpole (8, 9), but only 51–100% as efficacious in preventing goiter of the rat (10, 11). Using a cell-free fraction, it has been shown (12) that both DTP and TTA are about equivalent in affecting protein synthesis and in possessing less than half the potency of T_4. It will be interesting to determine how this cell line responds to some of the other well-characterized thyroxine analogs. Utilizing cultured cell systems may thus ultimately contribute to the elucidation of the relation molecular structure bears to hormonal activity and to the design of synthetic hormones possessing predicted actions and potencies.

Summary. The T-1 cell line, derived from the human kidney, responds to two active thyroxine analogs, diiodothyropropionic acid (DTP) and triiodothyroacetic acid (TTA), qualitatively paralleling findings made with other systems. Compared to thyroxine, DTP is about equivalent in stimulating cellular proliferation, but possesses only 0.1 its potency in augmenting colony formation by these T-1 cells; TTA is at least 10 times more potent. These observations extend the useful-

ness of the T-1 cell line as an *in vitro* model for characterizing the peripheral response to the iodinated thyroidal hormones.

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