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The influence of thyroid hormones and propylthioracil on salicylate hepatotoxicity in monolayer cell cultures*

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It has been shown in animal studies that propylthioracil (PTU) protects the liver from injury due to dietary deficiency [1, 2], carbon tetrachloride [3], alcohol [4] and acetaminophen [5, 6]. Based on the observation that normal thyroid function is necessary for complete expression of carbon tetrachloride injury [7, 8], the presumed mechanism of protection is inhibition of thyroid hormone synthesis. Further support for the concept that thyroid function modifies toxic liver injury is the observation that hyperthyroid animals have increased susceptibility to liver injury from anoxia [9], dietary deficiency [2, 10], chloroform [11], and infection [12, 13]. Recent studies have shown that PTU blocks the peripheral monodeiodination of thyroxine (T_4) to the more active triiodothyronine (T_3) [14–16]. This mechanism has been suggested as an explanation for the apparent protective effect of PTU [3].

Chronic alcohol administration in animals mimics the hypermetabolic state produced by excessive thyroid hormone [17]. PTU abolishes both this hypermetabolic state and the hepatic susceptibility to anoxic injury [4]. While development of a hypothyroid state may explain PTU suppression of liver injury, a more direct effect of PTU on the hepatocyte has not been excluded. Such an effect is suggested by evidence that alcohol-induced hepatic injury is suppressed by only 3 days of PTU treatment [4]. This does not appear to be consistent with thyroid hormone depletion in view of the prolonged effects of thyroid hormone [17] and the delayed onset of action of PTU in the treatment of hyperthyroidism.

This study was designed to determine the influences of PTU, T_4 and T_3 on salicylate-induced injury in hepatocyte monolayer cultures. By using such cultures, any effect of PTU would be independent of its effect on thyroid hormone synthesis. Furthermore, by studying T_3 alone any chemical effect of PTU would be independent of its affect on monodeiodination of T_4.

Methods and Materials

Hepatocyte monolayer cultures. Male Sprague–Dawley rats were subjected to subtotal hepatectomy under ether anesthesia and permitted to recover with free access to food and water. Four days later the regenerated livers were perfused using a modification of the method of Berry and Friend [18] and Bissell *et al.* [19]. The perfusate consisted of 0.01 M 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffered Hank's solution, pH 7.4, containing

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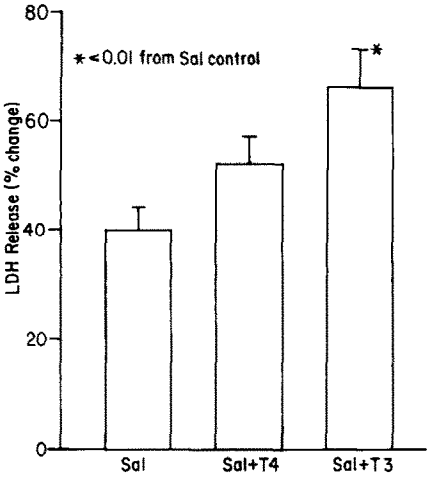

Fig. 1. Influence of thyroid hormone on salicylate hepatotoxicity. Results are expressed as means ± S.E.; N = 15.

0.5% crude collagenase (Worthington Biochemical Corp., Freehold, NJ). The cells were suspended in Eagle's Minimum Essential Medium containing 24 mM HEPES buffer and Hank's salts with glutamine (Grand Island Biological Co., Grand Island, NY); 200 mM gentamicin (Schering Corp. Kenilworth, NJ), 0.05 μg/ml, was added. Suspensions of 2.5×10^6 cells were placed in 60 × 15 mm plastic contour petri dishes and adjusted to a volume of 2.5 ml with medium. The cells were then incubated at 37° under 95% O_2 and 5% CO_2. Trypan blue (0.04%) exclusion was used to measure cell viability at the time of harvest, while visual inspection using an inverted microscope confirmed the continuity of the monolayer. After 24 hr the medium was changed and the cells were examined again for viability. Toxicity studies were then carried out. To eliminate between-culture variability and between-animal variability, each experiment was carried out on at least five culture plates from three different animals.

Toxicity studies. To select the concentration of sodium salicylate to be used in subsequent experiments, a dose–response relationship was first established. Lactate dehydrogenase (LDH) release, as previously described, was used as the measure of toxicity [20]. An optimum effect was obtained at a sodium salicylate concentration of 40 mg/dl which was used for subsequent experiments.

Hepatocytes were pretreated and incubated with equimolar (5.8×10^{-9} M) concentrations of either T_4 (*l*-thyroxine, sodium salt, Sigma Chemical Co., St. Louis, MO) or T_3 (3,3',5-triiodo *l*-thyronine, sodium salt, Sigma Chemical Co.) and/or PTU (6-*n*-propyl-2-thiouracil, anhydrous, Sigma Chemical Co.). These concentrations had been demonstrated in dose–response experiments (not shown) to produce the optimum enhancement of salicylate toxicity. After 24 hr, the media were changed, with sodium salicylate (40 mg/dl) being added to half the culture plates from each experimental group. The remaining control plates were reincubated with the pretreatment media. The hepatocytes were then incubated for another 4 hr. Following this, media were removed and passed through three micron Millipore filters prior to analysis for LDH.

LDH assay. LDH was assayed by the method of Wroblewski and LaDue [21] and expressed as percent change from control. This has been demonstrated to be a reliable measure of toxicity in hepatocyte culture systems [22]. The results were confirmed in a general way by microscopic inspection of the monolayer. Previous experiments confirmed that T_3, T_4, PTU and sodium salicylate had no effect on the LDH assay.

Statistical analysis. Values for LDH activity were converted to percent change from control, the control being 100%. Statistical significance was determined by the Wilcoxon rank sum test [23] and one-way analysis of variance.

Results

The influences of T_4 and T_3 on salicylate-induced injury are illustrated in Fig. 1. The addition of T_4 increased LDH release from 38.8 ± 4.3 (S.E.M.) to 52.0 ± 6.5%—a trend that was noted at several concentrations of T_4 but which never quite reached statistical significance. The addition of T_3 increased LDH release from 38.8 ± 4.3 to 66 ± 6.8% ($P < 0.01$). The presence of T_4 alone increased LDH release by 2.2 ± 4.2% (not significant), while T_3 increased LDH release by 14.1 ± 5.5% ($P < 0.01$) over control.

The effect of PTU on thyroid hormone-augmented salicylate injury is illustrated in Fig. 2. PTU appears to have protected the cells from both T_4- and T_3-augmented injury. The effect was dose related over a range of 10–60 μg/ml. PTU in the absence of T_4 or T_3 had no significant effect on salicylate toxicity. PTU alone did not affect LDH release from the cells.

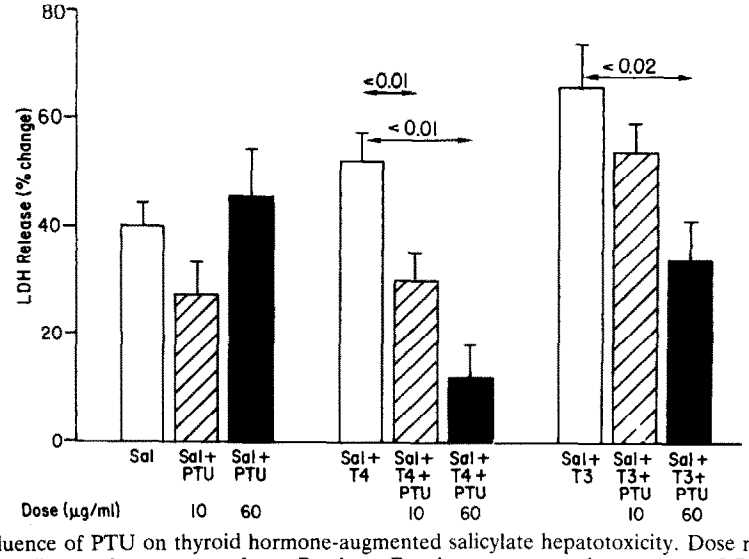

Fig. 2. Influence of PTU on thyroid hormone-augmented salicylate hepatotoxicity. Dose refers to the PTU dose. The numbers across refer to P values. Results are expressed as means ± S.E.; N = 15.

Discussion

T_3 augmented salicylate-induced injury to hepatocytes. This supports the observations of other workers who have used whole animals to demonstrate thyroid hormone-augmented injury due to alcohol [4] and carbon tetrachloride [3]. The reasons for this effect of thyroid hormone are not clear. T_3 is known to increase oxygen demand and respiration in the cell [24], but whether this is due to uncoupling of mitochondrial oxidative phosphorylation remains in doubt [25, 26]. Salicylates have been claimed both to uncouple mitochondrial respiration [27] and, particularly at higher concentrations, to inhibit mitochondrial respiration [28, 29]. Salicylate inhibition is thought to occur at the level of coenzyme Q and cytochrome c_1. The effect of thyroid hormone and salicylate at different levels of the respiratory chain would presumably be to severely inhibit mitochondrial respiration. This is a possible explanation for the apparent additive effect.

The data also demonstrate that PTU, in the presence of thyroid hormones, suppressed salicylate hepatotoxicity. This supports the observations in intact animals injured with alcohol [4] and carbon tetrachloride [3]. Since the suppression of salicylate toxicity was observed *in vitro*, the effect of PTU cannot be explained exclusively by inhibition of T_4 synthesis. Furthermore, PTU suppression occurred in the presence of T_3 alone and thus its effect cannot be explained entirely by inhibition of monodeiodination of T_4 to T_3. The possibility arises that PTU either inhibited or removed the end organ action of T_3 or interfered with salicylate toxicity by altering the uptake or metabolism of salicylate. Although there is some evidence that PTU may bind drug-metabolizing enzymes [27, 28], the fact that PTU had no effect on salicylate hepatotoxicity in the absence of thyroid hormones suggests that alterations in salicylate metabolism are an unlikely explanation for the effect of PTU.

The exact mechanism of PTU inhibition of toxic injury remains unknown, but its therapeutic application has been demonstrated in human alcohol-induced injury [30]. We have shown that the effect of PTU is, at least in part, independent of PTU's effect on T_4 synthesis and biotransformation. PTU thus may have an effect in addition to its inhibition of T_4 and T_3 synthesis. Furthermore, the possibility arises that the therapeutic benefit may be achieved without the undesirable side effect of hypothyroidism.

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