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Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS)☆

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Abstract

Introduction: Perfluorooctanesulfonate (PFOS) is widely distributed and persistent in humans and wildlife. Prior toxicological studies have reported decreased total and free thyroid hormones in serum without a major compensatory rise in thyrotropin (TSH) or altered thyroid gland histology. Although these animals (rats, mice and monkeys) might have maintained an euthyroid state, the basis for hypothyroxinemia remained unclear. We undertook this study to investigate the causes for the PFOS-induced reduction of serum total thyroxine (TT4) in rats.

Hypotheses: We hypothesized that exposure to PFOS may increase free thyroxine (FT4) in the rat serum due to the ability of PFOS to compete with thyroxine for binding proteins. The increase in FT4 would increase the availability of the thyroid hormone to peripheral tissues for utilization, metabolic conversation, and excretion. We also hypothesized that PFOS does not directly interfere with the regulatory functions of the hypothalamic–pituitary–thyroid (HPT) axis in rats.

Experiments: Three experimental designs were employed to test these hypotheses. (1) Female Sprague–Dawley (SD) rats were given a single oral dose of 15 mg potassium PFOS/kg body weight. At intervals of 2, 6, and 24 h thereafter, measurements were made for serum FT4, TT4, triiodothyronine (TT3), reverse triiodothyronine (rT3), thryrotropin (TSH), and PFOS concentrations, as well as liver PFOS concentrations, UDP-glucuronosyltransferase 1A (UGT1A) family mRNA transcripts, and malic enzyme (ME) mRNA transcripts and activity. (2) To provide evidence for increased uptake and metabolism of thyroxine (T4), ^{125}I-T4

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was given to male and female SD rats by intravenous injection, followed in 2 h by a single oral dose of 15 mg potassium PFOS/kg body weight. ^{125}I radioactivity was determined in urine and feces collected over a 24-h period and in serum and liver collected at 24 h. (3) To assess the potentials effect of PFOS on the hypothalamic–pituitary–thyroid axis, over an 8-day period, groups of male SD rats were given PFOS (3 mg/kg-d), propyl thiouracil (PTU, 10 μg/mL in water), or PTU and PFOS in combination, with controls receiving 0.5% Tween® 20 vehicle. On days 1, 3, 7, and 8, TT4, TT3, and TSH were monitored. On day 8, pituitaries were removed and placed in static culture for assessment of thyrotropin releasing hormone (TRH)-mediated release of TSH.

Results: (1) PFOS transiently increased FT4 and decreased TSH within 6 h, with values returning to control levels by 24 h. TT4 was decreased by 55% over a 24-h period. TT3 and rT3 were decreased at 24 h to a lesser extent than TT4. ME mRNA transcripts were increased at 2 h and activity was increased at 24 h. UGT1A mRNA transcripts were increased at 2 and 6 h. (2) ^{125}I decreased in serum and liver relative to controls and consistent with a reduction in serum TT4. Concomitantly, ^{125}I activity was increased in urine and feces collected from PFOS-treated rats. (3) During the 8 days of dosing with PFOS, TSH was not elevated in male rats, while TT4 and TT3 were decreased. Pituitary response to TRH-mediated TSH release was not diminished after 8-daily oral doses of PFOS.

Conclusions: These findings suggest that oral dosing in rats with PFOS results in transiently increased tissue availability of the thyroid hormones and turnover of T4 with a resulting reduction in serum TT4. PFOS does not induce a classical hypothyroid state under dosing conditions employed nor does it alter HPT activities.

Keywords: Perfluorooctanesulfonate (PFOS); Thyroid hormones; Liver; ^{125}I

1. Introduction

Perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$) has been found to be widely distributed in humans and wildlife (Butenhoff et al., 2006; Giesy and Kannan, 2001; Hansen et al., 2001; Harada et al., 2004; Houde et al., 2006; Martin et al., 2004; Olsen et al., 2003, 2004). PFOS is resistant to environmental and metabolic degradation, and it appears to accumulate in the food chain (Johnson et al., 1984; Olsen et al., 2005; Seacat et al., 2002). This finding has led to interests in environmental and health properties of PFOS at an international level (OECD, 2002).

Prior toxicological studies with PFOS have reported significant hypothyroxinemia that was characterized by decreases in total and free thyroid hormones in serum without a major compensatory rise in thyroid stimulating hormone (TSH) (Lau et al., 2003; Luebker et al., 2005; Seacat et al., 2002; Thibodeaux et al., 2003). These observations did not fit the usual diagnosis of hypothyroidism and were more similar to the profile of non-thyroidal illness syndrome, or NTIS (Chopra, 1997; Larsen et al., 2003; Ravel, 1995). In these studies, all of the thyroid hormones measured were performed using analog methods (with clinical laboratory autoanalyzers); however, Seacat et al. (2002) and Luebker et al. (2005) also demonstrated that free thyroxine (FT4) in PFOS-containing serum was unchanged compared to controls when a reference method (equilibrium dialysis followed by radioimmunoassay, ED-RIA) for FT4 measurement was used.

The discrepancy seen is due to the fact that analog systems use labeled thyroxine (T4) analog to bind with assay antibody in inverse proportion to the amount of competing FT4; and the amount of assay antibody-bound labeled T4 analog is measured. However, a certain amount of labeled T4 analog may be bound to endogenous and assay serum carrier proteins with varying degrees of affinity, and the assay calibration takes this into account. When a substance that effectively reduces serum carrier protein binding sites through competitive binding is introduced, such as PFOS or free fatty acids, less labeled T4 analog is bound than is normally accounted for in the assay calibration, forcing more labeled T4 analog toward assay antibody binding sites, resulting in an underreporting of FT4. The bias can be minimized with a reference method, ED-RIA. ED-RIA uses buffers that do not contain serum proteins so that binding interferences are greatly reduced.

As reported in our recent study (Chang et al., 2007), measurements of FT4 in serum containing PFOS by analog methods indeed are prone to negative bias due to displacement of bound T4 from carrier proteins in serum by PFOS. When PFOS was added to rat sera *in vitro* at concentrations ranging up to 200 μM, FT4 was shown to be increased up to 260% over paired control using ED-RIA but only 30% using a standard analog method. Furthermore, total thyroxine (TT4) remained unchanged.

A negative bias in the analog method for measurement of serum FT4 after PFOS exposures was also demonstrated *in vivo*. Twenty-four hours following the last of