Original Article

Assessment of Rodent Thyroid Endocrinology: Advantages and Pit-Falls*

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Abstract. In recent years, the number of commercially available rat hormone assays has increased and it is now feasible to include plasma hormone determinations as part of the toxicology safety assessment of novel compounds. This is especially true for the evaluation of the thyroid hormones. This article, which is a transcript of the lecture given to the First European Comparative Clinical Pathology Conference at Nottingham, April 1993, presents the advantages and pit-falls that an investigator must be aware of when assessing changes in plasma hormone concentrations on a toxicology study. In addition, the regulatory status of novel compounds that produce thyroid adenomas or adenocarcinomas in long-term oncogenicity studies, is discussed. Data are presented which show that the magnitude and consistency of a thyroid endocrine change, that can be expected following the administration of a compound that induces liver enzymes, is not as predictable as indicated by the literature. Hence changes in plasma thyroid hormone values may not be clearly demonstrated in rodents treated with compounds that produce thyroid tumours. This should not be of concern provided there is evidence of early follicular hyper trophy and an understanding of how the compound produces a change resulting in increased TSH drive on the thyroid gland (e.g. direct effect on the thyroid or an indirect effect via increased plasma clearance).

Keywords: Rats; Thyroid stimulating hormone; Thyroxin; Toxicology; Adenomas

Introduction

In regulatory drug safety evaluation more effort has been devoted to the assessment of the rodent thyroid hormone activity than to any other endocrine system. The reason for this interest lies in the observation that the rodent thyroid is particularly sensitive to drug-induced hormonal disturbance. The most common observation is an increase in thyroid stimulating hormone (TSH) secretion which, during chronic long-term studies, results in a sequential progression from normal thyroid follicular cells to cellular hypertrophy to follicular adenomas.

This paper considers the advantages of including thyroid hormone assessment as part of the safety evaluation of a novel compound, especially those that cause thyroid hyper trophy in subacute studies. The majority of the article will, however, describe the potential pit-falls of such an approach and will present experimental data to illustrate the difficulties of interpreting thyroid hormone endocrine changes.

Brief Review of Rodent Thyroid Endocrinology

Like all endocrine systems, the control of thyroid hormone synthesis and secretion is very precisely regulated. For an in-depth review of thyroid endocrine homocostasis the reader is directed to articles by Hill et al. (1989) and Thomas and Williams (1992). A very brief summary is presented in Fig. 1 which shows the key components of the pituitary – thyroid axis. The hypothalamus, which is situated behind the optic nerves at the base of the brain, secretes thyrotrophin releasing
hormone (TRH). This hormone stimulates the thyrotrophs within the pituitary gland to release thyroid stimulating hormone (TSH). As its name implies, TSH acts on the thyroid gland to stimulate the synthesis and release of the two thyroid hormones, thyroxine which contains four iodine atoms and is referred to as $T_4$, and tri-iodothyronine with three iodine atoms. In the circulation, both of these thyroid hormones are protein bound and less than 1% of these hormones circulate as free-$T_3$ and free-$T_4$. It is only the free hormones that are physiologically active and $T_3$, mainly produced by the deiodination of $T_4$, is about four fold more biologically active on target tissue nuclear receptors, than $T_4$.

Thyroid hormone homeostasis is regulated by TRH stimulation and modulated by a process of negative feedback whereby the level of circulating thyroid hormones directly influence pituitary TSH secretion. As the circulating levels of $T_3$ and $T_4$ fall there is less negative feedback on the pituitary, thus allowing the release of more TSH. This, in turn, stimulates the release of thyroxine resulting in higher $T_3$ and $T_4$ circulating values which inhibit further TSH secretion. The system is in dynamic equilibrium with the level of TSH secretion being largely dependent on the circulating levels of thyroxine and tri-iodothyronine.

It is very important to recognise that the concentration of circulating thyroid hormones depends not only on the rate of secretion from the thyroid gland but also on how quickly the hormones are removed from the circulation. Iodine atoms can be removed from $T_4$ by peripheral deiodination. Deiodination can occur at several sites but, from the plasma clearance aspect, of major importance are the kidney and the liver. $T_4$ may be deiodinated either by 5'-deiodinase to give the active hormone $T_3$, or by inner ring deiodination to form reverse $T_3$ (rT3) which has no biological function (Refetoff and Reed Larsen 1989). The liver can also conjugate the hormones by a process of glucuronidation and sulphation, resulting in the hormones being more water soluble which promote their excretion, via the bile, into the small intestine.

Sites of Drug Interference

As might be expected for such a complex control process, there are many sites for drug interference. Fig. 2 merely highlights a few important aspects of interest without providing a comprehensive review which is available elsewhere (Hill et al. 1989; Atterwill et al. 1992). In humans, circulating thyroxine is bound to a specific, high-affinity protein called thyroxine binding globulin (TBG). Because of this high affinity, thyroxine has a circulating half-life in man of about 7 days. In rodents, this protein does not exist and 75% of thyroxine is bound to albumin. This has a low affinity for thyroxine and, consequently, the half-life of $T_4$ in the rat...