Editorial: Deafness and Goiter: Molecular Genetic Considerations

Profound deafness is one of the most striking features of certain congenital thyroid diseases. In addition, lesser defects in auditory function, albeit of uncertain incidence and severity, have been described in acquired thyroid disorders in later life. Numerous reports in the medical literature have indicated a link between the function of the thyroid gland and the development and possibly the maintenance of function of the auditory system. Deafness can occur, for example, in sporadic cretinism or in congenital hypothyroidism in any given population. It is also common in a majority of the cases of neurological cretinism arising in geographical regions where endemic iodine deficiency prevails. It is perhaps ironic that, despite this clear association and the fact that severe deafness is an obvious symptom, we know so little about the mechanisms that connect the functions of the endocrine and auditory systems. It is only comparatively recently that unfolding events have indicated that progress is being made in understanding the underlying molecular mechanisms.

Deafness has long been associated with thyroid gland abnormalities. Indeed, this connection had been recognized before the identification of thyroid hormone, when the function of the thyroid gland still remained enigmatic. Reports dating from the latter half of the 19th century referred to deafness occurring in both sporadic and endemic cretinism and in a distinct condition of deaf-mutism with goiter, later termed Pendred's syndrome (1). The first hints that thyroid hormone was essential for auditory function followed the advent of replacement therapy with thyroid extracts, originally pioneered by G.R. Murray in 1891, which was later reported to be capable of improving the hearing as well as the growth and mental development of infants suffering from cretinism. The requirement for thyroid hormone has since been substantiated in animal models. A further intriguing link has been provided by study of the more recently described syndrome of resistance to thyroid hormone (RTH), which is associated with defects in the thyroid hormone receptor β (TRβ) gene. RTH is characterized by goiter and elevated levels of thyroid hormone but resistance of the pituitary and other organs to its actions (2). It is accompanied by a variable array of recognized symptoms, although these have generally not included deafness. However, as reported by Brucker-Davis et al. (4) in this issue of J.C.E.M (see page 2768–2772), an incidence of hearing loss is now revealed that may have escaped previous notice (3, 4). Despite the ostensibly similarity of congenital hypothyroidism, RTH, and Pendred's syndrome, in their association with deafness and goiter, it has become apparent that each has a distinct molecular basis.

Thyroid hormone and its receptors in the auditory system

While it is clear that thyroid hormone is required for the development of the auditory system it is imperfectly understood where, when, and how it acts. Classical studies by M.S. Deol (5) in the 1970's were among the first to suggest possible sites of action of thyroid hormone in the mammalian auditory system. Deol demonstrated that mice made congenitally hypothyroid by treatment with propylthiouracil (PTU) displayed cochlear deformities, which were interpreted as being severe enough to explain the associated deafness. It should be borne in mind that, while these and most subsequent studies have focused on the role of thyroid hormone in the cochlea, it may also be required in central auditory pathways in the brain, as has been suggested in the rat auditory cortex (6). However, the interpretation of any central defect is complicated by evidence that the correct development of central auditory pathways is dependent on sensory inflow, such that a defect might result as an indirect consequence of impaired cochlear function during development.

Further studies delineated a period of thyroid hormone dependency that coincides with a critical phase of morphological and functional development of the auditory system and suggests, therefore, that thyroid hormone is one of the major signals in control of this intricate developmental process. The use of PTU in combination with thyroxine replacement in mice and rats defined a period of thyroid hormone dependency, extending from midembryonic to early postnatal stages, corresponding approximately to human fetal development from the second trimester into the neonatal period (5). During this period, the rudimentary components of the cochlea undergo significant development, including the differentiation of precursor cell types into the hair cells and supporting cells in the organ of Corti, and the formation of the tectorial membrane and other structures necessary for the transduction of acoustic stimuli into nervous signals, which are conveyed by the spiral ganglion to the cochlear nuclei in the brainstem. Studies of hypothyroid rats and mice have revealed gross deformity of the tectorial membrane, hair cells, and fine structural defects in the hair cell stereocilia and in outer hair cell synapse formation (5, 7).

The requirement for thyroid hormone implicates the TR genes with a key role in converting the hormonal signal into a specific developmental response. The TR genes encode nuclear receptors, which act as hormone-dependent transcription factors and which bind and respond to the thyroid hormone tri-iodothyronine (T3). The TRs are versatile transcription factors, capable of both activation and repression of transcription. They have, therefore, the potential to regulate a sequence of downstream events necessary for cochlear...
development. The identification of two related TR genes, TRα and TRβ, which are differentially expressed, suggests that they mediate distinct aspects of the T3 response. Because both genes also differentially express splice variants, the response to T3 is likely to be subject to complex regulation mediated by a multireceptor family. In situ hybridization analysis of the rat inner ear has shown that both TRα and TRβ messenger RNAs are expressed in the cochlea (8), indicating that this is a direct site of action for T3. The TRα gene is also expressed in the vestibular structures, whereas TRβ is restricted to the cochlea, principally in the immature organ of Corti. Thus, both the TRα and TRβ genes are likely to play a direct role in cochlear development.

Studies of the DNA binding properties of the TRs have suggested that, so far, we may only be scratching at the surface in terms of understanding the transcriptional pathways regulated by the TRs. TRs bind stably to DNA in vitro as homodimers or as heterodimers with retinoid receptors or other members of the nuclear receptor family; in particular, the retinoid X receptor (RXR), which binds the ligand 9-cis retinoic acid, associates with the TR to form stable TR-RXR heterodimers on DNA. This points to a possible interaction of the TR with other signaling pathways that could not have been perceived from known thyroid physiology. Indeed, recent studies have shown that, in the embryonic mouse cochlea during the period of T3-dependence, the differentiation of hair cells is highly sensitive to retinoic acid (9). While the in vivo relevance of any interaction between the TR and retinoid pathways remains to be established, this should at least be considered in situations where both TRs and retinoid receptors may function, including in the auditory system.

Thus, a model for the occurrence of deafness in congenital hypothyroidism is that insufficient T3 during a critical embryonic and neonatal period causes a failure to activate the necessary TR-mediated transcriptional program in the cochlea and possibly at other central sites in the auditory pathway. While this is plausible, it should be noted that almost nothing is presently known of the target genes that are presumably regulated by TRs in the auditory system. Indeed, the identification and characterization of physiologically-significant target genes is central to understanding both the normal function of the thyroid hormone signaling pathway and its defective function that results in deafness. This important and challenging area is deserving of further study.

**Syndrome of resistance to thyroid hormone (RTH)**

While expression patterns have provided clues to where the TR genes may function, genetic analysis is required to provide direct evidence for the function of TRα and TRβ. RTH is associated with mutations in the TRβ gene. Indeed, there, already, served as a guide to the possible role for the TR genes in vivo. RTH is typically dominant, and it is associated with mutations that generate receptors with impaired response to T3, although they can still bind to DNA (10). In cotransfection assays, the TRβ mutants can inhibit normal transcriptional control by wild type TRs. Although the mechanism of inhibition is uncertain, it may involve dimerization with wild type receptors or competition for target DNA. Thus, a hypothesis has emerged whereby in a heterozygous individual who expresses one wild type and one mutant TRβ allele, the mutant receptor interferes with normal TR-mediated signaling pathways to cause the symptoms of RTH. Several questions, however, remain to be examined in this model. First, robust transcriptional inhibition in cotransfection assays generally requires a significant (5- to 10-fold) excess of mutant over wild type receptor. Therefore, this mechanism cannot simply be extrapolated to the in vivo situation, where these conditions do not occur. Secondly, in cotransfection assays, the mutant receptors act as pleiotropic inhibitors of transcriptional control by TRα, TRβ, or retinoic acid receptors, such that it is not clear which pathways may be inhibited by the mutant TRβ in vivo. On the strength of present evidence, it is not possible to correlate RTH phenotypes with specific transcriptional defects. There is clearly a need for additional assays of function, particularly those which address the role of the mutant receptors in the in vivo context.

Although profound deafness is not characteristic of RTH, a recent survey of 104 RTH patients revealed a 21% incidence of milder forms of hearing loss (3). Interestingly, there was also an increased frequency of ear, nose, and throat infections in RTH patients, and it was suggested that ear infections could explain the hearing loss in two-thirds of the cases, possibly reflecting defective immune responses or anatomical abnormalities in the ear. The authors have extended this study to investigate auditory defects in greater detail in 82 RTH patients in the young adult age group (4). Indicators of the function of middle ear (tiptometry, acoustic reflexes) and cochlea (otoacoustic emissions) suggested that 50% of the cases of hearing loss were attributable to conductive defects probably related to middle ear infections and 50% to sensorineural deficits in the cochlea. A further limited analysis indicated that central auditory functions in the brainstem were normal. The moderate 21% overall incidence may reflect the requirement for additional predisposing factors, which could be environmental or genetic, as genetic heterogeneity has previously been invoked to explain the variability of other symptoms in RTH (10). Thus, the mutant TRβ may increase the susceptibility of RTH patients to hearing loss, through both indirect and direct routes of inhibition of auditory function. An unexplored alternative is that in RTH the elevated levels of thyroid hormones acting through TRα may contribute to the defects because of the sensitivity of the immature auditory system to excessive as well as insufficient levels of thyroid hormone. Mouse models carrying dominant RTH-type mutations would be invaluable to investigate the possible mechanisms.

In one exceptional kindred, RTH has been observed to be recessive. RTH occurred in three of six children, and it was striking that the symptoms of all three included severe deafness (2). These cases harbor a large chromosomal deletion, which includes the TRβ gene, suggesting that in addition to its dominant etiology, RTH may result from the loss of TRβ (10). However, in view of the undefined extent of the deletion, which was large enough to remove several genes, and the consanguineous history of this kindred, which could lead to the expression of numerous recessive traits, it is difficult to ascribe any aspect of the symptoms to a single gene defect. Recently, however, knock-out mice have been generated that
lack specifically TRβ, and it was found that these mice exhibit a consistent, severe defect in auditory function (11). The mutant mice also display the dysfunction of the pituitary and thyroid glands, which is characteristic of both dominant and recessive human RTH (12). This demonstrates that TRβ is indeed essential for auditory function and suggests that its loss is likely to be the major cause of deafness in the human kindred with recessive RTI2.

Study of the knock-out mice has yielded further insights and a few more puzzles about the function of TRβ in the auditory system. Measurements of auditory-evoked brainstem responses in young mice confirmed that the requirement for TRβ for auditory function is developmental, consistent with TRβ expression in the embryonic and neonatal cochlea. This excluded the possibility that the defect was caused by late-onset, progressive hearing loss. However, in view of the previously described malformations in the cochlea in hypothyroidism, it was surprising that no such defects were found in mice lacking TRβ. This suggests a model whereby TRβ is responsible for fine structural or functional maturation of the auditory system, whereas TRα, or other unidentified receptors, mediate the overall morphogenesis of the cochlea. This model predicts that the various cell- and developmental stage-specific actions of T3 are facilitated by the differential expression and function of the two TR genes. Such a division of labor may explain in part how a single hormonal signal can elicit several complex stages of cochlear development. Further insights await the comparative study of auditory function in knock-out mice lacking TRβ, TRα, or both TRβ and TRα.

**Distinctions between dominant and recessive TRβ mutations**

Whereas the loss of TRβ consistently results in severe deficiency in auditory function, the typical dominant form of RTH only results in relatively mild hearing loss, which furthermore only occurs in a minority of cases. Thus, the consequences of recessive and dominant TRβ mutations on the auditory system are distinct in terms of severity, incidence, and therefore, probably also mechanisms of impairment. In contrast, both dominant and recessive TRβ mutations lead to a similar defect in the function of the pituitary-thyroid axis (10, 12), suggesting that in dominant RTH, the mutant receptor efficiently inhibits the residual wild type TRβ, producing a functional knock-out of TRβ in this system. However, since dominant mutations do not lead to the same severe deafness as the loss of TRβ, the mutant receptors in dominant RTH must be relatively impotent at inhibiting the essential function of the receptors encoded by the residual wild type TRβ allele in the auditory system. This suggests that, by as yet undefined mechanisms, the activity of TRβ is subject to differential tissue-specific modulation in the endocrine and auditory systems.

Another intriguing possibility is that, in dominant RTH, the T3-insensitive TRβ mutants may retain partial activity in the auditory system. This could reflect possible normal T3-independent functions for TRβ, as a growing body of evidence now suggests that the complex interaction of the TR with the DNA response element and the basal transcription machinery can occur in the presence or absence of T3, with the potential for different net outcomes on the transcription of the target (13 and refs. therein). These studies, based on cotransfection and in vitro transcription assays, suggest that the TR has both T3-dependent and T3-independent transcriptional control activities. This is remarkable, because it implicates the TR with additional functions independent of thyroid physiology, which could not have been predicted on the basis of the known actions of T3.

On a speculative basis, if it is assumed that there are T3-independent functions for TRβ in the auditory system, these may still be partially exerted by the T3-insensitive mutant receptor in dominant RTH. Recently, corepressor proteins have been isolated that interact with the TR in the absence of T3 to mediate T3-independent repression (13). Conceivably, such cofactors may still interact with the mutant receptor in dominant RTH whereas in the complete absence of TRβ, all T3-dependent and T3-independent functions would be abolished, which could provide a possible explanation for the severe phenotype caused by the loss of TRβ. Obviously, considerable study is necessary to investigate how the array of different TRs, RXRs, and cofactors, and their modulation by T3 are harmonized in vivo in the auditory system to give the necessary tissue and target gene-specificity.

**Pendred's syndrome and concluding remarks**

Pendred's syndrome is a recessive genetic disease, and it is one of the most common forms of inherited childhood deafness. The deafness is sensorineural and it is variably associated with cochlear malformation. Although accompanied by goiter, thyroid hormone levels are usually in the normal or slightly reduced range, such that the deafness cannot be caused by hypothyroidism. The organification of iodide into thyroglobulin is defective, leading to an accumulation of unincorporated iodide in the thyroid gland, suggesting that there is a defect in an enzyme required for the organification of iodide. Two recent genetic linkage studies have mapped the defect to chromosome 7, which ruled out the possibility that the mutation occurred in any known gene required for iodide organification, including thyroid peroxidase or thyroglobulin, which reside on other chromosomes (14,15). Incidentally, this also excludes direct involvement of the TRα or TRβ genes, which reside on chromosomes 17 and 3, respectively. These findings indicate that there is an as yet unknown step in the organification of iodide, which is defective in Pendred's syndrome.

It is unknown how such an enzyme defect in the thyroid gland might also cause deafness, and it has been suggested that there may be contiguous defects in two distinct genes in this region of chromosome 7. Interestingly, the defect localizes with an independently mapped gene, DFNB4, for autosomal recessive sensorineural hearing loss (14,15). Ultimately, fine mapping of this region should allow identification and cloning of the genes for Pendred's syndrome and DFNB4, which will resolve the puzzle of whether they are the same gene and whether Pendred's syndrome actually involves contiguous gene defects. It is also anticipated that this
will elucidate more clearly the step of incorporation of iodide into thyroglobulin in the biosynthesis of thyroid hormone.

Finally, it is worth broadening the perspective to mention studies of environmental contaminants that can interfere with thyroid physiology and auditory function. Polychlorinated biphenyls (PCBs) are persistent pollutants that bear structural similarities to thyroid hormone and that can interfere with the endocrine system. Embryonic and post-natal exposure to PCBs in rat development can reduce thyroid hormone levels and impair auditory function (16). Impairment is selective in the low frequency range, suggesting that apical regions of the cochlea that respond to low frequency stimuli are most sensitive to PCB-induced damage. This may reflect the fact that PCBs produce their greatest reduction in thyroid hormone levels during the later developmental period when the apex matures, whereas hormone levels are less reduced at earlier periods when the basal regions of the cochlea mature. These studies suggest that the major cause of the auditory deficiency is indirect, resulting from reduced levels of thyroid hormone, although the possibility that PCBs also act directly on the target tissue was not excluded.

These findings point to the potential contribution of environmental contaminants to human hearing loss. Because of the widespread distribution and bioaccumulative properties of PCBs, the global population is exposed to a greater or lesser extent, suggesting that further studies of the potential risks would be important. It would be ironic if a century of lesser extent, suggesting that further studies of the potential risks would be important. It would be ironic if a century of human scientific endeavour has made progress in elucidating the link between thyroid gland function and deafness in certain diseases, while at the same time, human industrial endeavor with its concomitant generation of toxic by-products, may have created new risks to the thyroid hormone signaling pathway.

In The Lancet in 1896, Pendred wrote, “The curious association of deaf-mutism and goitre occurring in two members of a large family has induced me to record these cases. Why this association? Perhaps some readers of The Lancet may be able to throw some light on the cause of this combination of diseases.” Presumably, Pendred expected to stimulate a rather immediate debate from contemporary readers. However, the question remains pertinent in 1996 despite a century of studies on the link between various thyroid disorders and deafness. Perhaps the difference today is that not only can the question be debated but, with the benefit of modern techniques in genetics and molecular biology, it can be mentally investigated. There are grounds to believe that advances in understanding may follow.

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