Activating mutations of TSH receptor

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Mutations activatrices du récepteur TSH

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Ann. Endocrinol., 2003 ; 64, 1 : 12-16

Les mécanismes d’activation du récepteur TSH coupé à sa protéine G seraient dépendants d’une libération des contraintes structurelles permettant le récepteur « relâché » d’activer la protéine G. Par analogie aux travaux expérimentaux sur le récepteur adrénergique α1b montrant que les mutations peuvent créer une activation constitutive du récepteur, il est proposé que de semblables mutations somatiques spontanées du récepteur TSH peuvent être à l’origine d’adénomes thyroïdiens toxiques. Cette hypothèse est confirmée. Par ailleurs, il est démontré que de rares cas d’hyperthyroïdie familiale non autoimmune sont l’expression de mutations des cellules souches du récepteur TSH. Il en est de même, pour des cas d’hyperthyroïdie néonatale non autoimmune. Outre l’activation constitutive du récepteur TSH, la sensibilisation du récepteur TSH à l’hCG par mutation du domaine extracellulaire est identifiée comme cause de l’hyperthyroïdie gestationnelle familiale. Ces différentes études de mutation contribuent à notre connaissance des mécanismes d’activation des hormones glycoprotéiques. Le premier modèle était proposé d’après les données de ces mutations. Selon ce modèle, le domaine extracellulaire du récepteur a une action inhibitrice sur le domaine transmembranaire de telle sorte qu’il faut interrompre l’interaction pour permettre l’activation du récepteur. Mais les résultats expérimentaux les plus récents suggèrent que l’interaction entre le domaine extracellulaire et le domaine transmembranaire est plus complexe qu’une simple inhibition ; il est possible qu’à l’activation, le domaine extracellulaire passe d’une structure inhibitrice à une structure activatrice.

Mots-clés : Thyroïde, récepteur TSH, mécanisme d’activation, grossesse.

Marcogher et al. have demonstrated, in vivo, the pathogenic role of the TSH-R by stimulating antibodies in Graves’ disease, or can be observed spontaneously in autonomous thyroid toxic adenomas.

Ledent et al. [15] have demonstrated, in vivo, the pathogenic role of a continuous production of cAMP in the thyrocytes, by targeting the adenosine A2 receptor in these cells under the control of the thyroglobulin.

Key words: Thyroid, thyrotropin receptor, mutation, activation, hypersensitivity, pregnancy.

BACK-GROUND

The TSH receptor (TSH-R) is a member of the large family of G protein-coupled receptor [1]. Based on bacteriorhodopsin tridimensional structure, then on rhodopsin, those receptors are thought to be organised in seven transmembrane domains, linked by extracellular and intracellular loops. The TSH-R, LIICG and FSH receptors, define a subfamily of G protein-coupled receptors, because of a very large extracellular domain [20]. This extracellular domain is known as the ligand binding domain [16, 20]. The intracellular loops of the receptor are the structural basis for coupling to trimeric G protein, and subsequently to the production of second messenger.

In the human thyroid cells, the TSH-R is coupled to Gsα and Gq proteins, and subsequently to the cAMP and inositol phosphates pathways [2, 27]. Activation of the cAMP leads to proliferation of the thyrocytes, as well as to differentiation, ie expression of thyroperoxidase, thyroglobulin, Sodium-Iodide symporter, ...

Abnormally sustained high cAMP production can be due to stimulation of the TSH-R by stimulating antibodies in Graves’ disease, or can be observed spontaneously in autonomous thyroid toxic adenomas.
promoter in transgenic mice. Animals were affected by a huge life threatening hyperfunctioning goiter.

**CONSTITUTIVE ADRENERGIC RECEPTORS AS A MODEL**

The experimental work of the group of Lefkowitz on the adrenergic receptor can be regarded as pioneer for understanding of physiology of G protein-coupled receptors. By introducing artificial mutations in the third intracellular loop of the α1b adrenergic receptor, this group observed a production of Inositol triphosphate, the second messenger, in the absence of the natural ligand [13]. The receptor had turned autonomous and independent, or constitutively activated. Furthermore, it appeared that mutation of a particular residu in the third intracellular loop, the Alanine 293, in each of the possible aminoacids, each time had the same effect: constitutive activation of the receptor. A new model for activation of G protein-coupled receptors was proposed [23]. In this model, receptors are thought to exist spontaneously in at least two different conditions. The unactivated or silent state, with maximal structural constraints, and the activated, relaxed state, with minimal structural constraints. The receptor spontaneously oscillates between the two extreme states. In the absence of the ligand, the majority of the molecules of receptors are maintained in the constrained conformation (inactive receptor), whereas the ligand through it’s binding to the receptor favours displacement of the equilibrium toward the relaxed conformation (activated receptor).

The mutation of the adrenergic receptor is then thought to activate the receptor by relieving the structural constraints, and the alanine 293 then appears as the keystone of the structural constraints.

Because the spatial organization was supposed to be similar for the different G protein-coupled receptors, and mechanisms of activation thought to be shared, it was hypothesized that same cause would have the same effects in the TSH-R. Activating mutations of the TSH-R were then looked for.

**SOMATIC ACTIVATING MUTATIONS IN THYROID TOXIC ADENOMAS**

The initial verification of the hypothesis came from the study of toxic autonomous thyroid adenomas [17]. The autonomy of the tumor, demonstrated by the hypercapitation-organification, of radioactive iodide on scintiscan, contrasting with extinction of the rest of the thyroid gland, and associated with a blunted TSH, is a capital characteristic of the condition. It ensures that the mechanism of hyperactivity of the nodule is an intrinsic feature of the tumor rather than being caused by any extra tumoral stimulus (TSH, TSH-R antibodies).

Genomic DNA was extracted from the tumor and used as a template for amplification of part of the coding sequence of the TSH-R gene. This amplified DNA could then be sequenced, and mutations could be found in the third intracellular loop, as expected, particularly a mutation of the residu corresponding to Alanine 293, the Alanine 623 [17, 23]. The heterozygote mutations present in the tumor, were absent of the peripheral blood as well as normal thyroid tissue surrounding the adenoma.

However, in following studies, surprisingly, many mutations were described, scattered throughout the whole transmembrane domain [4, 7, 18, 19, 21]. No functional map of the TSH-R could really be designed according to these results.

*In vitro* experiments confirmed the constitutive activation of the mutated TSH-R, with cAMP accumulation, in absence of TSH, higher for the mutant than for the wild-type receptor, in spite of a lower expression. By contrast with this constant cAMP hyperproduction, activation of the diacylglycerol-inositol phosphate pathway is not common. The response to bovine TSH is usually conserved, with an increased apparent affinity for bovine TSH.

The frequency of activating mutations of TSH-R as cause of toxic adenomas has been controversial. Some authors found mutations only in a few cases, whereas frequencies as high as 50 to 82% were reported by others [4, 7, 18, 19, 21, 26]. It appears, that in european countries, where borderline to moderate iodine deficiency is common, those mutations are the major cause of toxic adenomas. Interestingly, in a few cases, different somatic mutations were found in different independent toxic nodules in the same multinodular goiter [5].

**GERMLINE ACTIVATING MUTATIONS IN FAMILIAL NON AUTOIMMUNE HYPERTHYROIDISM**

Since somatic mutations activating the receptor could lead to a focal autonomous growth, and hyperfunction, it was postulated that the same kind of mutation present in each of the thyrocyte would lead to a diffuse hyperfunctionning goiter. This would be easily achieved by a germline mutation, and would lead to a hereditary disease. The phenotype had been described earlier, in the early eighties, by Thomas et al. [25] who reported a...
family in which hyperthyroidism was observed, across three generations. This hyperthyroidism was associated with a diffuse goiter, in the absence of Graves’ ophthalmopathy. The blood tests failed to detect any kind of thyroid antibodies, especially TSH-R antibodies. Finally, pathology of thyroid tissue for those of the patients that went through thyroidecotomy, did not find any sign of an autoimmune process.

The same germline mutation of the TSH-R receptor was found in all affected members of this family, as well as another mutation was found in another unrelated family [3]. A higher constitutive activity of the mutated TSH-R was demonstrated here again, by comparison with the wild-type receptor.

Several additional pedigrees have been published since this first publication, and in all cases hypertyroidism transmits as an autosomal dominant trait (reviewed in [4]). Although the condition is rare, the diagnosis should not be missed, because one of the characteristic is the frequent relapse of goiter and hyperthyroidism in absence of an aggressive ablative therapy. Furthermore, identification of such cases allows for presymptomatic diagnosis, and consequently early treatment, provided a careful follow-up has been established.

**SPORADIC NON AUTOIMMUNE NEONATAL HYPERTHYROIDISM**

Some cases of neonatal hyperthyroidism have been reported in which no evidence of autoimmune thyroid disease, either in the neonate or in the mother could be demonstrated. Hyperthyroidism, in these cases is usually severe, and requires intensive and immediate antithyroid therapy. A goiter is the rule. In one case [1], exophthalmia was reported, without clear explanation. In these cases, germline activating mutations of TSH-R have been demonstrated also [11, 14]. Since they were not found in the genitors, they are neomutations.

**FAMILIAL GESTATIONAL HYPERTHYROIDISM DUE TO HYPERSENSITIVITY TO HCG**

Normal early pregnancy is marked by a moderate increase of free T4 with a subsequent decrease of plasmatic TSH, around the 10-12 weeks of gestation [8]. This increase in thyroid hormones is explained by the thyrotropic activity of hCG due to structural homologies between hCG and TSH. These hormones are built as heterodimers associating a common α subunit and different β subunits responsible for the specificity of the hormone. Although different, the β subunit have some homologies also, allowing for some cross-stimulation of the three different receptors for glycoproteic hormones, namely the TSH-R, LH/CGR-R and FSH-R [10]. These cross-stimulations are usually not observed at physiological concentration of the hormones, except during pregnancy where hCG reaches concentrations high enough to stimulate modestly the TSH-R. The moderate hyperthyroxinemia, contemporary of the pic of hCG secretion, is of short duration, and does not require any treatment since clinical signs are usually absent, and when present rapidly resumes after hCG concentration has dropped down. True hyperthyroidism can be rarely observed, sometimes requiring transient antithyroid drug treatment. These cases are usually observed when hCG secretion is abnormally important or sustained, and hyperthyroidism is associated with, or presenting as, hyperemesis gravidarum [9].

We had the opportunity to study an atypical case of gestational hyperthyroidism, not caused by any autoimmune disease, nor by a toxic adenoma. Recurrence of hyperthyroidism and hyperemesis gravidarum in each pregnancy, contrasted with normal hCG level, required antithyroid drug treatment during the whole pregnancy. Interestingly, the patient’s mother had suffered the same condition during each of her pregnancies.

A heterozygous mutation of the TSH-R was found in the patient and her mother, changing Lysine 183, in the extracellular domain, into an Arginine. Hypersensitivity of the mutant TSH-R to hCG was demonstrated in vitro [22].

**IS THERE ANY CORRELATION BETWEEN GENOTYPE AND PHENOTYPE?**

One recurrent tentation when dealing with pathogenic mutation in human disease, is to try to correlate genotype and phenotype, with the hope there could be a kind of genetic prognosis following the genetic diagnosis.

When sorting the activating mutations of the TSH-R according to their potency, an interesting observation was the apparent higher constitutive activity of most of somatic mutations when compared with germline hereditary mutations affecting different residues. In fact, it is known an autonomous adenoma has to reach a critical size before it leads to hyperthyroidism. This can lead to the hypothesis that the potent somatic mutations lead to a more severe phenotype, as represented by a stronger stimulation of proliferation. These mutations if transmitted as germline mutation would lead to severe in utero and neonatal hyperthyroidism, and would then be lethal. In agreement with this hypothesis, the observation that germline neomutations...
of the “adenoma type” in congenital hyperthyroidism, lead to extremely severe features requiring immediate therapeutic.

However, there is, at least, one exception to this “rule”. In a family affected by hereditary non autoimmune hyperthyroidism, a mutation already encountered in toxic adenomas was observed. The phenotype, was not especially severe, since hyperthyroidism was diagnosed in children after several years of life [12]. On the other hand, a correlation between genotype and phenotype would be suggested by this observation, since in this family, mitral valve prolapse cosegregated with the mutation and hyperthyroidism. The relationship between the two features is, however, far from clear.

If recognition of the genetic nature of familial non autoimmune hyperthyroidism can allow for presymptomatic diagnosis and a mandatory follow-up of the mutation carriers this can certainly not predict who will become hyperthyroid and when, neither how severe the hyperthyroidism will be. In some families, several asymptomatic carriers were identified and the age at onset of hyperthyroidism was not uniform [3, 25]. Genotype is therefore hardly correlated to phenotype.

A MODEL OF TSH-R ACTIVATION?

In structural studies, directed mutagenesis is a potent tool, allowing for identification of functional domains of a receptor or its ligand. On the other hand, study of natural mutations avoids a bias in the selection of residues to be mutated and focus on mutations associated with a phenotype, thus affecting residues presumably important for function.

In the case of TSH-R, an intriguing observation has been the diversity and the spatial dispersion of activating mutations. This observation made it difficult to delineate specific domains as the activation domains. Mutations located in the extracellular domain, and a deletion in the third extracellular loop [6, 19] suggested that the inhibitory constraints in the model of G protein-coupled receptor could be exerted by the extracellular domain on the transmembrane domain. This was recently demonstrated by the production of truncated TSH-R lacking the extracellular domain, and exhibiting a higher constitutive activity than the wild-type receptor [28]. However, additional data obtained in this model showed that relief of these structural constraints was not sufficient for a full activation of the receptor, and suggested that the extracellular domain has to switch from an inhibiting to an activating conformation when TSH binds it.

The precise binding sites of TSH, although already studied through extensive mutagenesis, are still poorly determined. The broadening of specificity associated with a very mild mutation of a conserved residue such as the lysine 183 into Arginine, is somehow surprising. New data [24] suggest that rather than directly interacting with the ligand, would it be TSH or hCG, the Lysine 183 interacts with neighbouring residues involved in the binding of the ligand.

CONCLUSIONS AND PERSPECTIVES

The search for activating mutations of the TSH-R has been triggered by previous studies on other G-protein coupled receptors. It seems quite clear now, that no direct and clear correlation between the genotype and the observed phenotype can be found. However the identification of germline activating or sensitizing mutations is of clinical value, especially for the therapeutic management and the risk of relapse of the disease. In contrast, diagnosis of somatic activating mutations in toxic adenomas is of no help to the clinician.

However, description of such a large panel of activating mutations, scattered throughout the whole receptor, has been of great help to the searcher. A proposed model for activation of TSH-R by disruption of putative inhibitory interactions between the extracellular domain and the extracellular loops has got some basis with these natural mutations. This model, however, appears to be too simple, and has to be complexified. The structural basis for ligand binding and for specificity are still poorly defined also, and progress is expected to come with description of additional mutations sensitizing TSH-R to hCG that may be more frequent than expected. The functional dichotomy between the extracellular domain (binding site) and the transmembrane and intracellular part (transduction domain) now appears as an oversimplistic model for TSH-R and related receptors.


