PUTATIVE MOLECULAR PATHOGENESIS OF FELINE HYPERTHYROIDISM – SIFTING THROUGH THE SIGNAL TRANSDUCTION CROSSTALK

Richard K. Burchell B.Sc. (Hons) B.V.Sc., MMedVet (SAM) DECVIM-CA (SAIM)
Senior Lecturer, Small Animal Medicine, Institute of Veterinary and Biomedical Sciences
Massey University, Palmerston North, New Zealand

Introduction
Feline hyperthyroidism (FH) is an endocrinopathy characterised by an autonomously hyper functioning thyroid that produces thyroid hormones in a disorderly manner¹. Since FH was first described, there has been a steady increase in the number cases diagnosed and FH is now the most common feline endocrinopathy¹. Notwithstanding the salient observation that FH may have increased in prevalence due to more rigorous surveillance, the preponderance of expert opinion, suggests that FH is a relatively recent entity within the feline population². Notwithstanding intense research efforts, the aetio-pathogenesis of FH remains elusive. Unlike man, in which Grave’s disease is the most common cause of hyperthyroidism³, in cats the disease resembles toxic nodular goitre (TNG), which has been described in people secondary to a number of causes, including G protein mutations, amiodarone use, iodine fluctuations and a number of other goiterogenic drugs and toxins⁴.

Studies have identified risk factors associated with FH including, indoor lifestyle, canned food diet polybrominated diphenyl ethers, use of a litter box and use of flea treatment¹,²,³,⁴. In addition, fluctuating iodine levels have been implicated in the pathogenesis of FH²,³,⁶-⁷, which is further supported by the observation of iodine induced hyperthyroidism in people who develop hyperthyroidism after being supplemented with iodine following a period of iodine deficiency⁵. In spite of all of these efforts, FH cannot as yet be experimentally reproduced by any of the ostensible risk factors, and therefore a plausible molecular explanation for the genesis of an autonomously functioning thyroid gland has not yet been described.

Research efforts to date have focussed on the identification of risk factors associated with the suspected genesis of FH, and subsequent exploration of these factors from a molecular perspective in an attempt to elucidate the molecular aetipathogenesis of FH, which will inform preventative therapies/strategies for FH. The author contends that these studies have directed research questions and efforts, and somewhat hindered the establishment of research questions based on other epistemological paradigms. It would appear that a few highly interesting thought experiments pertaining to FH have not been investigated in the literature. For example, why dogs and in particular, small breed dogs, which are presumably exposed to many of the risk factors implicated in FH, do not develop TNGs. In addition, why do cats and a subset of human patients develop TNG, which is not a common disease entity in other species? It is unlikely that environmental factors alone are responsible for the apparent rise in prevalence.

Normal regulation of thyroid hormone synthesis
Regulation of the thyroid hormone axis (THA) is elegantly controlled by feedback mechanisms, which are well understood in endocrine physiology. Thyroid hormones (TH) are liberated in response to thyroid stimulating hormone (TSH) secreted by the anterior pituitary, which is a G-protein coupled second messenger system, resulting in the activation of the sodium-iodide (NaI) symporter. The NaI symporter utilises the sodium gradient generated by the Na/K ATPase to drive organic iodine into the cytoplasm of the thyroid follicular cell. Thyroid stimulating hormone secretion is regulated by thyroid releasing hormone (TRH), which is produced in the hypothalamus in response to a complex, incompletely understood, plethora of endocrine and neurological stimuli. Indeed, the elegant sophistication of the THA lies in its ability to orchestrate and fine-tune metabolic and physiological processes according to the environmental and homeostatic needs.

The eventual elaboration of the TH is a classic example of signal transduction in molecular physiology, and it is within this mechanism that the pathogenesis of TNG is most likely to occur. The TSH receptor is a G-protein coupled transmembrane protein, which activates adenylate cyclase following activation, resulting in the production of cyclic adenosine monophosphate (cAMP). The production of cAMP results in the activation of protein kinase A (PKA) that in turn phosphorylates many target proteins and enzymes. In most cases phosphorylation results in activation or increased activity of target proteins, but in some cases the b-form of the enzyme (the non-phosphorylated form) is the more active. Consequently, the cAMP/PKA response is able to simultaneously augment and attenuate certain target pathways.

Importantly, the signal transduction cascade precipitated by TSH signalling results in expression of a number of key genes, namely, the sodium/iodide (NaI) symporter, thyroperoxidase (TPO) and thyroglobulin (TG).
Therefore, activation of the TSH cascade, results in a massive influx of iodine into the cytosol of the thyrotrophs. Iodine is transported in to the follicular lumen by pendrin and an unidentified thyroid carrier. The regulation of this process is incompletely understood, however DUOX2 and TPO appear to play a role in the efflux of iodine. Once iodine arrives in the follicle it is incorporated into tyrosine residues, which are abundant on the thyroglobulin molecule. There are three key steps in the organification of iodine. Firstly, iodine must undergo peroxidation prior to incorporation into tyrosine. This redox reaction requires H₂O₂ as substrate which is generated by DUOX2 on the apical membrane of the thyroid follicle cell. The peroxidation of iodine is catalysed by thyroperoxidase, which serves as the priming step for iodine organification. Oxidised iodine is highly reactive, and non-specifically iodinates tyrosine residues on TG. Tyrosine has 6-carbon phenol ring side-chain, which has 2 potential binding sites for iodine namely, the 3' and 5' binding position. Peroxidised iodine binds in a random and non-specific manner to tyrosine, resulting in moniodothyrosine (MIT) or diiodothyrosine (DIT). Thyroperoxidase then catalyses the coupling of MIT and DIT tyrosine residues bound to thyroglobulin, resulting in the production thyroxin (T₄) triiodothyronine (T₃) and reverse T₃. This process results in a mixed soup on thyroglobulin of MIT/DIT/T₄/T₃ and rT₃. Thyroglobulin, therefore serves as a reservoir, for preformed TH metabolites.

Thyroid hormone loaded thyroglobulin is liberated from the colloid follicular lumen, in an endocytic vesicle in a process that is regulated by cAMP/PKA mediated phosphorylation, and thus is under the regulation of TSH. These TH containing vesicles walk along the cytoskeleton and are destined to fuse with lysosomes, forming polyphagolysosomes. Inside the lysosome, proteolytic cleavage emancipates thyroid hormones and MIT and DIT, which are released into the cytosol. TH and its intermediates are prevailed upon by deiodinases which denudes MIT and DIT of their iodine ions, which are subsequently re-circulated, and which produce T₃, and rT₃ which are minor TH release products. The predominate TH product is thus T₄.

The synthesis and release of TH is intricately orchestrated by the signal transduction cascade evoked by TSH. The key TSH mediated regulatory points in the genesis and secretion of TH are:
1) Increased iodine uptake by increased expression of NaI
2) Increased expression of TG and TPO, both of which are involved in iodine efflux, organification and TH production and,
3) The released of TH-laden TG from the thyroid follicle.

Thyroid hormone synthesis and release is thus exquisitely and tightly regulated by TSH. Of particular interest is the facultative expression of the NaI which is the first step of TH synthesis, which is induced by TSH signalling. Attenuation of NaI expression is associated with a decrease in the uptake, organification and release of thyroid hormones. The importance of NaI in the regulation of TH synthesis is emphasised by the fact that one of the most rapid mechanisms of reducing TH production in response to physiological stimuli, is a reduction in NaI expression and activity.

Molecular mechanisms associated with the pathogenesis of hyperthyroidism

In Grave’s disease, which is the most common form of human hyperthyroidism, antibodies mimic the action of TSH and results in sustained dysregulated production of TH by up regulating the steps mentioned above. The functioning of the thyroid is thus under the influence of TSH albeit through cunning molecular mimicry, which remains a physiologically appropriate regulatory process. Intense research scrutiny and a number of elegant experimental mouse model experiments have excluded a Grave’s like syndrome in the pathogenesis of FH. Interestingly, FH thyroid glands are able to function autonomously and are orphaned from the influence of TSH. In FH iodine is incorporated into TH in a continual and dysregulated manner. Therefore, enzymes normally facultatively expressed, in a proportional manner commensurate with physiological demands, become constitutively expressed, in what must surely be a molecularly fascinating conundrum.

Currently there are two main models of human hyperthyroidism that resemble FH namely, amiodarone induced hyperthyroidism and iodine induced hyperthyroidism or the Jod Basedow phenomenon. Iodine’s effects on thyroid hormone synthesis are of particular interest, given the well-described Wolff-Chaikoff effect and the ability of iodine to change gene expression. The Wolff-Chaikoff (WC) effect is the reduction of TH synthesis that occurs in the face of iodine excess, and serves to blunt the increased production of TH as a result of increased substrate independent of the action of TSH. Several molecular mechanisms have been proposed to explain the WC effect including decreased activity of peroxidases and decreased H₂O₂ production. The

---

1 Iodine organification refers to the incorporation of inorganic iodine into tyrosine residues on thyroglobulin.
purpose of the WC is to prevent sudden spikes in TH due to changing iodine levels. Following the acute WC effect, an escape from the WC effect is observed.\textsuperscript{16} This escape mechanism occurs in spite of persistently increased iodine levels, and serves to restore the reduction in TH due to the WC effect. This escape mechanism is incompletely understood and appears to be related to decreased expression of the NaI symporter. In addition, NaI levels also fall independently of mRNA levels, suggesting post-transcriptional or post-translational mechanisms, which appear to precede a decreased expression of NaI. Resultantly, less iodine is imported, in the face of elevated levels, which attenuates the WC effect in the cytosol by reducing the amount of iodine imported.\textsuperscript{16,22} This sophisticated molecular system ensures a smooth production of TH that is independent of fluctuating iodine levels, and thus ensures that TH production does not subvert the thyroid axis control mechanism.

Consequently, having comprehended the regulation of an exquisitely elegant physiological system, a number of interesting questions arise. Firstly, how does NaI activity circumnavigate the stringent dominance of TSH signalling? As mentioned the expression of NaI is facultative, and is continually calibrated by TSH, such that removal of the pituitary results in hypothyroidism. Molecularly therefore, the hyper functioning toxic goitre must overcome two tiers of control in the synthesis of TH, namely, the associated decrease in TSH that ensues with increased TH, and abrogation of the WC effect, when an appropriate amount of iodine is absorbed. One possibility is that like in the case of certain tumours, that the TSH receptor (TSHR) becomes activated, and fails to extinguish its signal transduction, once the physiological need has abated.

G-protein mutations
To this end, because TSHR is a G-protein coupled protein, and because G proteins can be both stimulatory and inhibitory (G\textsubscript{i}/G\textsubscript{o}), aberrant G protein signalling has been implicated in the pathogenesis of TNG. In humans a number of gain-of-function TSHR activation mutations have been identified. Interestingly, however, not all patients with these mutations develop hyperthyroidism. In cats, the findings of studies pertaining to G protein expression in TNGs has been seemingly contradictory, with evidence to support\textsuperscript{22-24} and refute\textsuperscript{25} the role of G proteins in FH. Anomalous continual G protein signalling would satisfy the requirements of the thyroid in circumvent the protective mechanisms of TH secretions, by augmenting the effect of TSH without the need for cell surface activation. In addition, the alpha G protein \textit{Vibrio cholera} toxin is an egregious example of the potential cellular devastation of dysregulated G protein signalling. However, the G protein paradigm fails to explain the variability in the development of FH and also the apparent geographical variations. In addition, the action of enzymes such as phosphodiesterase serve to abrogate the mechanism of the cAMP second messenger system. Lastly, the late age of onset of FH is somewhat perplexing within the G protein paradigm, and it is unclear what would drive gain-of-function mutations, unless the mutation is always present, and the development of FH represents the eventual physiological counter-regulatory fatigue associated with relentless signalling.

Endocrine disruptors
A plethora of endocrine disruptors have been implicated in the pathogenesis of FH including but not limited to bisphenol A, polybrominated diphenyl ethers (PBDE) and soy isoflavones. Molecularly, these compounds antagonise the action of TH by interfering with deiodonases, or displacing TH from receptors, thus attenuating the expression of the thyroid response elements (TRE).\textsuperscript{2} Purportedly, this results in decreased feedback on the pituitary, with increased TSH signalling causing the development of a goitre. Ostensibly, aberrant signalling of the THA is an attractive theory in the genesis of an autonomous goitre, but it cannot escape attention, that this mechanism is in stark contrast to the conclusions of the seminal work on autonomous goitres that demonstrate the thyroid’s complete independence from requisite pituitary support that is the normal situation.\textsuperscript{14} It must be remembered, that all of the enzymes involved in the uptake, organification, and release of TH are under the tight regulation of the TSH signal transduction pathway. Therefore, perpetual thyroid synthesis represents an unremitting activation of cAMP signalling, as well as increased expression of the NaI symporter in a non-facultative manner, or under the influence of a different gene regulatory element. This author contends that elucidating the regulation of the promoter/operator of the NaI gene will significantly advance our understanding of the pathogenesis of FH and perhaps the TNG of human hyperthyroidism. Its seems plausible that a number of proteins/factors are involved in the regulation of NaI expression, in much the same was as occurs in the regulation of parathyroid hormone synthesis.

\textsuperscript{2} TREs are a composite set of genes that regulate the response of thyroid hormone, and which are activated by TRE signaling.
Iodine
The role of iodine in the pathogenesis of FH remains ambiguous and elusive\textsuperscript{1,2,4,5,26–28}. Subsequent to the initial assessment of iodine requirements in kittens, there has been considerable discussion as to the iodine levels of feline diets\textsuperscript{2}. Discovery of the Wolff-Chaikoff effect has exemplified the potential role iodine may play in the pathogenesis of FH, by demonstrating the ions effect on the molecular machinery of the thyrocyte\textsuperscript{18,19,21,29,30}. In addition, cats fed lower iodine diets appear to be predisposed to the development of hyperthyroidism\textsuperscript{2}. In humans, iodine supplementation in individuals with a chronic iodine deficiency, has been known to induce non-Grave’s disease hyperthyroidism\textsuperscript{29,30} in a process that has been termed the Jod-Basedow phenomenon, and is considered the opposite of the normal Wolff-Chaikoff effect. Iodine’s ability to change the activity of enzymes, and the expression of the Na\textsuperscript{+} symporter\textsuperscript{11} and the synthesis of TH make it a potential candidate in altering the molecular architecture and gene expression patterns of the thyrocyte, which is strengthened by the potential risk-association that has been described. However, several aspects of iodine’s potential role remain unresolved, since iodine excess in man is associated with both TNG and Hashimoto’s hypothyroidism, indicating that the pre-existing molecular anomalies exist that alter the effect of fluctuating iodine. It would appear that iodine in iodine deficient individuals is more likely to result in hyperthyroidism, whereas in iodine replete individuals, it is more likely to cause hypothyroidism. In addition, individuals genetically predisposed to thyroid auto-immunity appear to be at risk of iodine associated hypothyroidism, which has also been observed in amiodarone-associated hypothyroidism (see below). In addition, the observation that many cats consume similar diets, but on a subset develop FH seems to exclude iodine as the sole cause of FH, and most opinion leaders feel that iodine alone is too simplistic to explain FH\textsuperscript{1,2,4,26}. Whilst iodine alone may not explain fully explain the pathogenesis of FH, unlocking its ability to alter gene expression is sure to provide useful insights into non-TSH mediated regulation of key TH genes.

Amiodarone
Whilst thyroid disease manifestations are relatively uncommon in patients treated with amiodarone, in a certain cohort of patients two types of thyroid disease are possible, namely hyperthyroidism and autoimmune hypothyroidism (AIH)\textsuperscript{51–56}. Amiodarone appears to function similarly to iodine in terms of eliciting disease in man, where those predisposed to AIH appear to develop hypothyroidism, whilst individuals with pre-clinical non toxic goitres appear to succumb to the effects of the Jod-Basedow phenomenon. It would be tempting to speculate that the disease process with amiodarone is similar to that of iodine, and thus improving our understanding of this mechanism may prove invaluable in determining the molecular aetio-pathogenesis of FH.

Concluding remarks
Whilst the unfortunate conclusion of this report is that the molecular aetiology of FH remains elusive, it becomes clear that a number of thought experiments, and unpacking the mechanisms of gene expression in the thyrocyte, will go a long way to unlocking the mystery of FH, and possibly TNG in man. In addition, given the inevitable redundancy of physiological pathways, as exemplified by the RAAS-escape phenomenon in ACE inhibitor therapy, it is highly plausible, that overlapping alternate signal transduction pathways, under different regulation systems are involved in TH. The recent discovery of TSHR on certain cancers, and demonstration of the effects of cytokine induced signal transduction, would appear to bolster the wisdom of this view. The author and his colleague’s hope to molecularly explore some of these pathways in naturally occurring disease in the target species, thereby abrogating some of the limitations of experimental models.

References
8. Unknown. escape from wolff chaikoff effect.pdf.