Identification, quantification, and *in vivo* effects of Thyronamine Analogues of Catecholamines in Sprague Dawley Rats

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Background

Thyroid hormones (TH) are essential endogenous amino acids that have critical genomic and non-genomic roles in the body. THs consist of two active forms, triiodothyronine (T3) and thyroxine (T4), and one inactive form, reverse triiodothyronine (RevT3), that can be further decarboxylated into the category of compounds known as thyronamines (TAMs). Formerly, the two known TAMs were 3-iodothyronamine (3-T1AM) and, the less potent but fully deiodinated, thyronamine (T0AM). Previously, the adult brain was thought to be thyroid-hormone insensitive [1], however, these TAMs have been so far confirmed to be in rodents' brain, liver, heart and blood [2]. Interestingly, TAMs induce physiological effects that counteract those initiated by THs when injected *in vivo* (Fig. 2) [3]. The biosynthesis of TAMs remains controversial; however one proposed pathway suggests that 3-T1AM is generated by sequential deiodiation by deiodinase (DIO), selenoenzymes and decarboxylation by aromatic L-amino acid decarboxylase (AADC) [4] (Fig. 3).

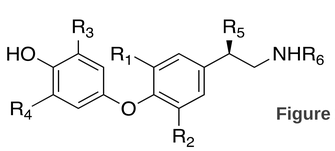


Figure 1. Basic structure of thyronamines.

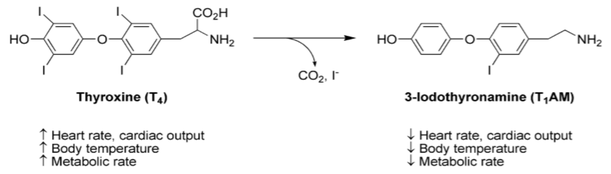


Figure 2. Comparison of the physiological effects induced by T4 and 3-T1AM [3].

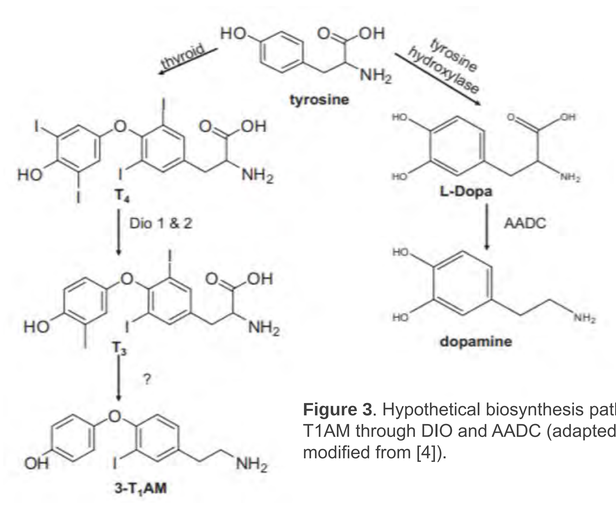
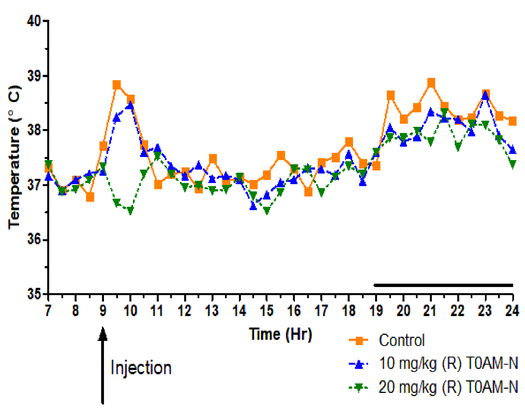


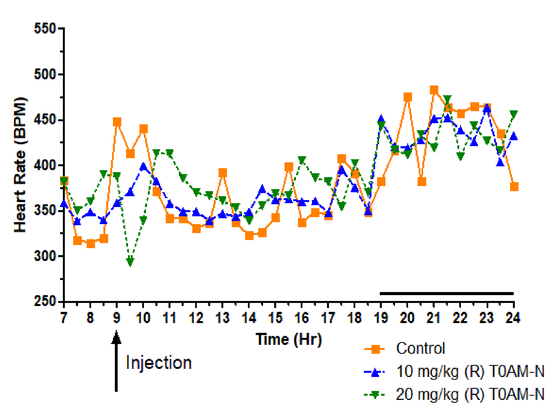
Figure 3. Hypothetical biosynthesis pathway of 3-T1AM through DIO and AADC (adapted and slightly modified from [4]).

Hypothesis

We propose that there are other undiscovered TAMs that can induce neurophysiological effects on the body, resembling adrenergic actions and TAMs that can counter them as well.

Results





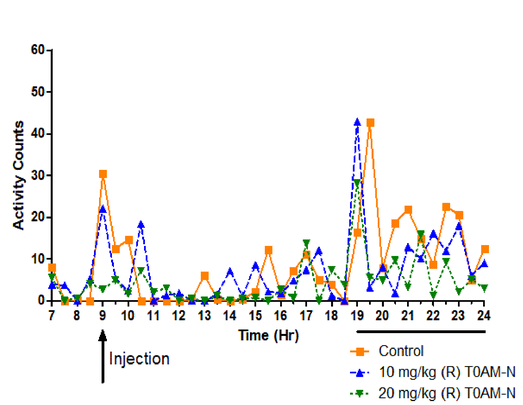
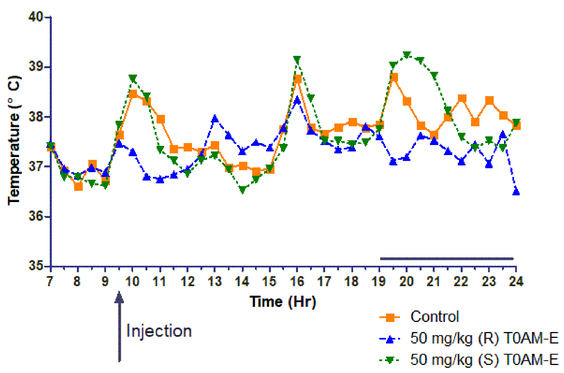


Figure 4. Physiological effects of T0AM-N. Normalized core body temperature (left), heart rate (middle) and motor activity (right) in rats. Control rats were injected with vehicle solution of 0.9% NaCl + 0.001 N HCl solution, while experimental group received similar solution with dissolved T0AM-N. Data was recorded over a 19 hr period from 7am to 12 am. The dark line from 19 – 24 hrs indicates the rat's dark phase**.** n=2.



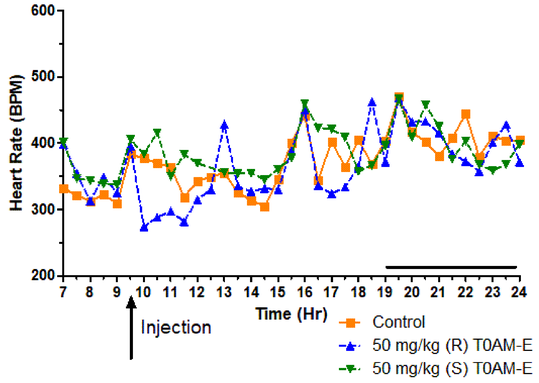


Figure 5. Physiological effects of R and S isomers of T0AM-E. Normalized core body temperature (left) and heart rate (right). in rats. A decrease in core body temperature and heart rate was observed post-injection in 50 mg/kg (R) T0AM-E group. n=2.

Methodology

Physiology of TAMs in vivo

- A telemetry device (PDT 4000 HR E-Mitters) was surgically implanted into adult Sprague Dawley rats. Banamine (0.4%) was subcutaneously injected into rats post-surgery and bacitracin was applied to the incision area.
- Rats were given 4-6 days to recover from surgery and were monitored for possible post-surgical complications.
- Rats were weighed a day prior to injections.
- Data collection started at 7am the day before injections using the VitalView software.
- Intraperitoneal (IP) Injections were administered at 9am.
- Data collection was stopped at least 19hrs after injections and analyzed using Excel and GraphPad Prism.

TAM in Adrenal Glands and Synaptosomes

- Sprague Dawley rats were sacrificed, and the brain and adrenal glands were harvested.
- Synaptosomes were prepared from the cerebral cortex.
- Adrenal glands were dissected into adrenal medulla and cortex.
- Tissue samples were extracted and analyzed using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and Bruker Daltonics Data Analysis 4.3 software.

Conclusions and Future Work

IP injections of T0AM-N *in vivo* have no effect on physiology
Stereochemistry of TAMs (R vs. S configuration) can result in different physiological effects *in vivo*

Future Work/Primary Focus

- Analysis of TAMs in adrenal glands and synaptosomes

Acknowledgments

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