3-iodothyronamine (T1AM): an endogenous antagonist of muscarinic receptors endowed of bronchodilatory activity

A. Laurino[1], M.E. Manni[1], G. Vistoli[2], R. Matucci[1], L. Raimondi[1]

[1] Dept. of NEUROFARBA, Section of Pharmacology, School of Human Sciences, University of Florence, Italy
[2] Dept of Pharmaceutical Science, University of Milan, Italy

3-iodothyronamine (T1AM) is a trace amine derived from thyroid hormone metabolism circulating in rodents and humans (Scanlan et al 2004; Galli et al., 2012; Saba et al., 2010., Manni et al., 2013) whose physiological role remains to be discovered. Much is instead known on T1AM pharmacological effects including hyperalgesia and stimulation of learning in mice (Manni et al.2014). The mechanisms responsible for such effects are only partially exploited (Laurino et al., 2015, in presss). T1AM pharmacodynamics features include interaction at trace amine type 1 (TAAR1), TAAR5, alfa2 and beta2 adrenergic receptors (Scanlan et al., 2004; Regard JB et al., 2007; Meyer T and Hesch RD, 1983). Interestingly, at such receptors the amine behaved as an agonist, as antagonist but also as inverse agonist. More recently, Khajavi et al. (2015) reported T1AM as a modulator of TRPM8, thus suggesting T1AM as a multi-target molecule and as a possible modulator of G-protein coupled receptors.

We aimed to investigate whether this amine, and some of its congeners, were able to interact at cholinergic receptors and, if it was so, if this interaction could produce any functional consequences.

We evaluated the 1) binding capacity of a series of thyronamines on nicotinic and muscarinic receptors (mAchR) of mice brain cortex and on human muscarinic receptors (hmAchR1-5) stable transfected in CHO cells; 2) whether interaction of T1AM at mAchR3 modified basal or Ach-induced pERK levels by western blot, 3) the conformational profile of T1AM at mAchR3 by MonteCarlo simulations. The lowest energy structures generated were then utilized in docking simulations involving three available mAChR resolved structures. Finally, T1AM effect on bronchoconstriction was evaluated in isolated Guinea pig trachea and in anaesthetized rats.

Our results indicate that Thyronamines bound to muscarinic and not to nicotinic receptors. In particular, thyronamine (T0AM), the 'basic not iodinated structure' recognized hmAchR1-5. Instead, T1AM and 3,5 di-iodiothyronamine (T2AM), preferentially recognized mAchR3 and mAchR2 respectively. Interestingly, T1AM did not stimulate basal pERK levels but rather it decreased, concentration-dependently, Ach-induced pERK1/2 levels in CHO cells transfected with hmAchR3. To assess any functional anti-muscarinic effects of T1AM we verified whether T1AM reduced carbachol-induced bronchoconstriction in isolated guinea pig trachea and in bronchi preparations from rats. Our results indicated that T1AM reduced carbachol-induced constriction in Giunea pig trachea (EC50 8 microM). Moreover, intratracheal administration of T1AM (0.4, 1.32 and 4 microg/kg) reduced carbachol-induced bronchoconstriction in rats with a maximum effect at 1.32 microg/kg.

Thyronamines interact at muscarinic but not at nicotinic receptors. Among the amines, T1AM the one showing a preferential binding for hmAchR3 showing an affinity 30 times higher than for hmAchR2, the cardiac subtype. Interestingly, T1AM behaves as an antagonist at mAChR3. In line with this T1AM reduced carbachol-induced constriction in isolated tracheas with a same order of potency observed in binding experiments (EC50 8 microM). Instead,T1AM potency in reducing bronchoconstriction *in vivo* resulted much higher than that measured in isolated trachea and in respect of its pKi vs. mAchR3. These findings indicate T1AM a physiological modulator of muscarinic receptors, a finding increasing the knowledge on T1AM pharmacodynamics features. However, the mechanisms responsible for the difference in T1AM potency between in vivo/in vivo effects remains to be investigated.

Thyroid patients often present diseases related to dysfunction of the cholinergic system, including anti-muscarinic manifestations. The mechanism underlying this co-morbidity is largely unknown. We propose accumulation of thyroid hormone metabolites, including T1AM, as a possible pathogenic event.