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3-Iodothyroacetic acid (TA_1), a by-product of thyroid hormone metabolism, reduces the hypnotic effect of ethanol without interacting at GABA-A receptors

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ABSTRACT

3-iodothyroacetic acid (TA_1) is among the by-products of thyroid hormone metabolism suspected to mediate the non-genomic effects of the hormone (T3).

We aim to investigate whether TA_1 systemically administered to mice stimulated mice wakefulness, an effect already described for T3 and for another T3 metabolite (i.e. 3-iodothryonamine; T1AM), and whether TA_1 interacted at GABA-A receptors (GABA-AR).

Mice were pre-treated with either saline (vehicle) or TA_1 (1.32, 4 and 11 μg/kg) and, after 10 min, they received ethanol (3.5 g/kg, i.p.). In another set of experiments, TA_1 was administered 5 min after ethanol. The latency of sleep onset and the time of sleep duration were recorded. Voltage-clamp experiments to evaluate the effect of 1 μM TA_1 on bicuculline-sensitive currents in acute rat hippocampal slice neurons and binding experiments evaluating the capacity of 1, 10, 100 μM TA_1 to displace [^{3}H]flumazenil from mice brain membranes were also performed.

4 μg/kg TA_1 increases the latency of onset and at 1.32 and 4 μg/kg it reduces the duration of ethanol-induced sleep only if administered before ethanol. TA_1 does not functionally interact at GABA-AR.

Overall these results indicate a further similarity between the pharmacological profile of TA_1 and that of T_1AM.

1. Introduction

Among endogenous occurring thyroid hormone (T3) derivatives is 3-iodothyroacetic acid (TA_1), produced by alternative metabolism of T3 involving decarboxylases, amine oxidases and deiodinases activities. We demonstrated recently that TA_1, in the central nervous system of mice, can be produced by the oxidative deamination of endogenous but also pharmacologically administered 3-iodothyronamine (T_1AM) carried on by monoamine oxidases (Laurino et al., 2015a). This finding suggested that the biosynthetic relationship between TA_1 and T_1AM might have physio but also pharmacological relevance, implying the two metabolites might reproduce similar effects when administered to rodents. Experimental evidence indicate that it might be not so.

We reported previously that TA_1 pharmacologically administered to mice at low doses induced itch, reduction of noxious heat threshold (Laurino et al., 2015b) and, as T_1AM, it stimulated memory acquisition and reverted scopolamine-induced amnesia (Musilli et al., 2014). Interestingly, TA_1, as T_1AM, induced these effects showing a wide range of dose effectiveness and inverted U-shaped dose response curves. On the contrary, Hoefig et al. (2015) reported evidence indicating that TA_1 administration, differently from T_1AM, did not induce hypothermia, bradycardia or modification of feeding behavior.

Abbreviations: GABA-AR, GABA-A receptor; TA_1, 3-iodothyroacetic acid; T_1AM, 3-iodothyronamine; T3, thyroid hormone.

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We believed important to explore further on possible similarities or differences between the pharmacological effects of T_1AM and TA_1 in order to understand which drug should be used to expect a certain effect. To this aim we investigated whether TA_1 was able to induce, as already reported for T_1AM (and T3), mice wakefulness (James et al., 2013) and whether TA_1 interacted at GABA-A receptor (GABA-AR) which is among the master regulators of drowsiness, sedation and sleep.

To explore these points we studied the effect of administering 1.32, 4 and 11 μg/kg TA_1 to mice prone to sleep for receiving an ipnotic dose of ethanol while the interaction at GABA-AR was studied electrophysiologically and by binding experiments.; **Abbreviations:** **3**-iodothyroacetic acid (TA_1); 3-iodothyroanmine (T_1AM); 3,3′,5,5′-tetra-iodothyroacetic acid (TETRAC); triiodothyroacetic acid (TRIAC), GABA-A receptor, GABA-AR; Thyroid hormone, T3.

2. Methods

2.1. Animals

All animal experiments were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 80-23, revised 1978). All efforts were made to minimize the number of animals used and their suffering. For this study we received the permission from the Ethical Committee for animal health (#959/2015-PR) from the Italian Ministry of Health.

A total of 80 male mice (CD1 strain; 20–30 g) from ENVIGO (Italy) were used. The animals were kept at 22 ± 1 °C with a 12 h light–dark cycle (light on at 07:00 h) and were fed a standard laboratory diet with water *ad libitum*. Five mice were housed per cage.

Wistar rats 15-to28-day old from Envigo (Italy) were used for preparation of acute hippocampal slices. Rats were sacrificed by decapitation under anesthesia with isoflurane and used for the preparation of acute organotypic hippocampal slices.

All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Ethanol-induced sleep

2.2.1. Experimental setting 1

Mice were pretreated with either saline or TA_1 (1.32, 4 and 11 μg/kg) and, after 10 min, they received an hypnotic dose of ethanol (3.5 g/kg, i.p.).

2.2.2. Experimental setting 2

Mice received a hypnotic dose of ethanol (3.5 g/kg, i.p.) and, after 5 min, they were treated with saline or TA_1 (1.32, 4 and 11 μg/kg, i.p).

In experimental *setting 1*, the time of latency for onset of sedation, defined as the inability of mice to right themselves when positioned on their back (LORR latency) and the time to regain it (i.e. the time elapsed between the onset of sedation and the righting of mice back onto all four paws, known as the LORR duration) were recorded. Recovery was defined as the time at which mice could right themselves three times in 30 s after being placed on their backs. In experimental setting 2 only the LORR duration was recorded.

Results are presented as the mean ± SEM of measures from 10 different mice.

2.3. Preparation of acute rat hippocampal slices

Brains were removed and mounted in the slicing chamber of a vibroslicer (Leica VT 1000S). Horizontal slices (250 μm in thickness)

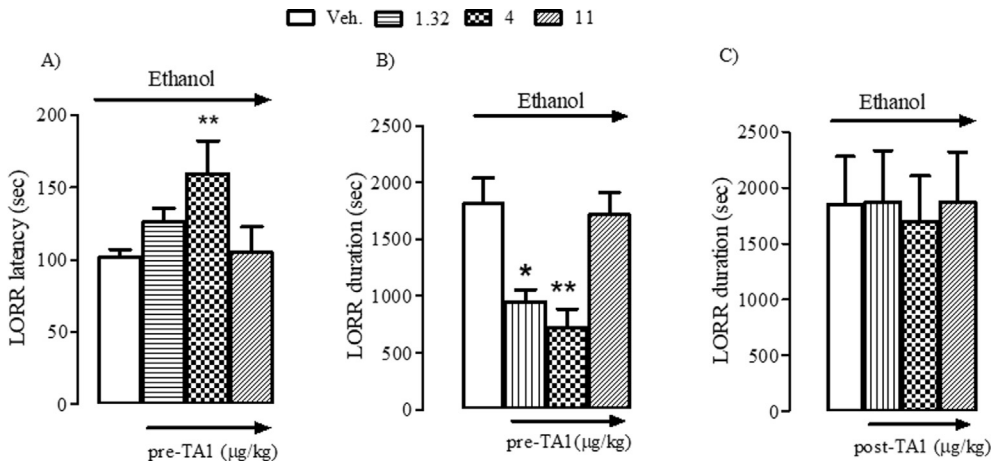

Fig. 1. TA1 reduces the LORR latency and LORR duration of ethanol-induced sleep.
Mice were treated with saline (Veh) or with 1.32, 4 or 11 μg/kg TA_1 and after 10 min (*setting 1*) or just after they received ethanol (3.5 g/kg; i.p., *setting 2*). The time of onset (*setting 1*) and the duration of sleep (*setting 1 and 2*) was measured as described in "Methods".
Panel A: the effect of TA_1 given before ethanol (pre-TA1) on LORR latency.
Panel B: the effect of TA_1 given before ethanol (pre-TA1) on LORR duration.
Panel C: the effect of TA1 given after ethanol (post-TA1) setting 2 on LOOR duration.
Results are expressed as the means ± SEM of measures relative to 10 mice. *$p < 0.05$ vs. Veh; **$P < 0.01$ vs. Veh.

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