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RESEARCH PAPER

Histamine mediates behavioural and metabolic effects of 3-iodothyroacetic acid, an endogenous end product of thyroid hormone metabolism

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BACKGROUND AND PURPOSE
3-Iodothyroacetic acid (TA1) is an end product of thyroid hormone metabolism. So far, it is not known if TA1 is present in mouse brain and if it has any pharmacological effects.

EXPERIMENTAL APPROACH
TA1 levels in mouse brain were measured by HPLC coupled to mass spectrometry. After i.c.v. administration of exogenous TA1 (0.4, 1.32 and 4 μg·kg^{-1}) to mice, memory acquisition-retention (passive avoidance paradigm with a light-dark box), pain threshold to thermal stimulus (51.5°C; hot plate test) and plasma glucose (glucorefractometer) were evaluated. Similar assays were performed in mice pretreated with s.c. injections of the histamine H_1 receptor antagonist pyrilamine (10 mg·kg^{-1}) or the H_2 receptor antagonist zolantidine (5 mg·kg^{-1}). TA1 (1.32 and 4 μg·kg^{-1}) was also given i.c.v. to mice lacking histidine decarboxylase (HDC$^{-/-}$) and the corresponding WT strain.

KEY RESULTS
TA1 was found in the brain of CD1 but not of HDC mice. Exogenous TA1 induced amnesia (at 0.4 μg·kg^{-1}), stimulation of learning (1.32 and 4 μg·kg^{-1}), hyperalgesia (0.4, 1.32 and 4 μg·kg^{-1}) and hyperglycaemia (1.32 and 4 μg·kg^{-1}). All these effects were modulated by pyrilamine and zolantidine. In HDC$^{-/-}$ mice, TA1 (1.32 and 4 μg·kg^{-1}) did not increase plasma glucose or induce hyperalgesia.

CONCLUSIONS AND IMPLICATIONS
Behavioural and metabolic effects of TA1 disclosed interactions between the thyroid and histaminergic systems.

Abbreviations
T_3, tri-iodothyronine; TA1, 3-iodothyroacetic acid; TA1M, 3-iodothyronamine; TRIAC, tri-iodothyroacetic acid

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Introduction

The main pathways of tri-iodothyronine (T_3) metabolism are deiodination of the aromatic rings, decarboxylation or conjugation of the alanine chain, producing the corresponding amines and esters, and transamination or oxidative deamination yielding acetic or propionic acid derivatives. The individual steps of this complex metabolism require cell-specific enzyme expression and can occur in different combinations, generating a plethora of compounds bearing amino acidic, amine and acidic moieties, with different degrees of iodination.

While decarboxylation was classically believed to follow deamination, it has recently been observed that thyronamines, lacking the carboxyl group and retaining the amine group, are endogenous compounds and at least some of them, in particular 3-iodothyronamine (T1AM), should be regarded as chemical messengers (Scanlan *et al.*, 2004; Saba *et al.*, 2010). T1AM has been detected in plasma of mammals (Hoefig *et al.*, 2011; Galli *et al.*, 2012) and its levels increases in pathological conditions including diabetes (Galli *et al.*, 2012). Acidic and amine derivatives are likely to differ in terms of their pharmacodynamic properties. For instance, while tri-iodothyroacetic acid (TRIAC) is an endogenous antagonist at thyroid hormone receptors, thyronamines exert rapid effects activating G-coupled membrane receptors (Zucchi *et al.*, 2006) and possibly interfering with membrane transporters or mitochondrial proteins (Cumero *et al.*, 2012).

TRIAC is not the only acid metabolite of T3, as 3-iodothyroacetic (TA1) and thyroacetic acids (TA0) may be produced by subsequent deiodination of TRIAC or by oxidative deamination of T1AM and thyronamine (T0AM) respectively. In the presence of deiodinases, TA0 can also be produced. TA1 was recognized as the main oxidative metabolite of T1AM (Wood *et al.*, 2009; Saba *et al.*, 2010; Agretti *et al.*, 2011), while TA0 was identified in human urine as the final end product of thyroid hormone metabolism (Pittman *et al.*, 1972). In turn, TA1 can be transformed into T0A, in cells expressing deiodinase activity.

We recently described some pharmacological effects of low doses of T1AM, including stimulation of memory acquisition and retention, increase of plasma glucose and reduction of pain threshold in mice. These effects occurred within 15 min after injection and were prevented in animals pretreated with clorgyline, an inhibitor of MAO activity (see Alexander *et al.*, 2013a). Moreover, clorgyline pretreatment also increased the systemic bioavailability of T1AM given i.c.v. (Manni *et al.*, 2012). These findings suggested that generation of TA1 by deamination might contribute, at least in part, to the acute effects of T1AM *in vivo* and showed that oxidative deamination had a marked effect on T1AM pharmacokinetics Overall, it can be hypothesized that T1AM, TA1, and possibly TA0, should be considered as part of the large family of thyroid hormone metabolites with potential biological activity.

Here, we have investigated whether endogenous TA1 could be found in mouse brain We have also evaluated potential signalling activity of exogenous TA1 in the CNS, by assessing its effects on memory, pain threshold and plasma glucose. The mechanism of action of TA1 was explored by using histamine receptor antagonists (see Alexander *et al.*, 2013b) and by examining its effects in mice lacking histidine decarboxylase ($HDC^{-/-}$) and the corresponding WT mice.

Methods

Animals

All animal care and experimental procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80-23, revised 1996) and were approved by the Animal Care Committee of the Department of Pharmacology, University of Florence, in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS no. 123) and the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering. Studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). A total of 200 animals were used in the experiments described here.

Male mice (CD1 strain; 20–30 g) from Harlan-Nossan (Italy) were used. Animals were kept at 23 ± 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. Mice lacking histidine decarboxylase ($HDC^{-/-}$) and the corresponding WT strain ($HDC^{+/+}$) on a 129/Sv background (18–22 g) were a kind gift from Dr Ohtsu, Japan (Ohtsu *et al.*, 2001). These animals were housed, four to six to a cage, in standard transparent laboratory cages. Cages were placed in the experimental room, 24 h before tests to allow adaptation.

Detection of the HDC gene. The genotypes of the knockout mice used with respect to the HDC gene were confirmed at birth, by amplifying the DNA prepared from tail biopsies by PCR, as described by Anaclet *et al.* (2009).

Detection of endogenous TA1 and T1AM in brain

Four mice from each of the CD1, $HDC^{+/+}$ and $HDC^{-/-}$ strains (20–30 g) were killed by cervical dislocation. The brains were isolated and quickly frozen at –80°C. TA1 and T1AM were then assayed by HPLC coupled to tandem mass spectrometry, as described previously (Saba *et al.*, 2010), with the only difference that tissue was homogenized with ultrasound, extracted with acetonitrile and then washed with hexane to remove lipids

I.c.v. injection procedures

I.c.v. injection was performed under light ether anaesthesia according to the method described by Haley and McCormick (1957) with minor modifications. The depth of anaesthesia was checked by monitoring respiratory rate (which was reduced within 2 min) and testing the lack of pain response to gentle pressure on the hind paws. The head of the anaesthetized mouse was grasped firmly and the needle of a 10 µL microsyringe (Hamilton Bonaduz, Bonaduz, Switzerland) was

inserted perpendicularly 2 mm through the skull into the brain. Ten microliters of solution were then slowly injected (in 20 s) into a lateral ventricle. The injection site was 1 mm to the left from the midpoint on a line drawn through to the anterior base of the ears. Immediately after needle removal, the animal remained quiet for approximately 1 min and then resumed its normal activity. To ascertain that solutions were administered exactly into the cerebral ventricle, some mice were injected with 10 µL of 1:10 India ink and their brains were examined macroscopically after sectioning. 95% of the i.c.v. injections were found to be correctly delivered.

The passive avoidance paradigm: the light–dark box

The test was performed according to the step-through method described by Jarvik and Kopp (1967). The experimental apparatus consisted of a two-compartment acrylic box with a brightly lit compartment connected to a dark compartment by a guillotine door. The dark chamber was constructed with a pitfall floor. When entering this chamber in the training session, mice receive a non-painful 'punishment' consisting of a fall (from 40 cm) into a cold water bath (10°C). Because mice prefer dark to the light, they would usually enter the dark compartment within 5 s. Mice not entering the dark compartment within 60 s during the training session were excluded from the experiment. The test was then repeated 1 and 24 h after the training session. Each pharmacological treatment was performed before the training session. In particular, mice received an i.c.v. injection of 10 µL of vehicle (Veh; 0.5% DMSO) or of TA1 (0.4, 1.32 and 4 $\mu g \cdot kg^{-1}$; n = 20 for each treatment) and, after 15 min, the training session was performed.

In other sets of experiments, mice were pretreated with i.p. scopolamine (0.3 $mg \cdot kg^{-1}$) or saline; with the histamine H_1 receptor antagonist pyrilamine (10 $mg \cdot kg^{-1}$, s.c.) or the histamine H_2 receptor antagonist zolantidine (5 $mg \cdot kg^{-1}$; s.c.) or saline. 15 min later, they all were injected i.c.v. with Veh or TA1. In all cases, the training session was performed 15 min after injecting TA1, by placing mice on an illuminated platform (60 W, 840 lux) and allowing them to enter the dark compartment. The extent of 'punishment' memory was expressed as the time (s) spent in the light portion during the training and the retention sessions. Retention sessions were performed 1 and 24 h after the training session. In the 1 and 24 h tests, each animal was placed on the platform and the time taken to enter the dark compartment (latency) was measured up to a maximum of 300 s.

Nociceptive thresholds to thermal stimulus using the hot plate test

Mice received i.c.v. TA1 (0.4, 1.32 and 4 $\mu g \cdot kg^{-1}$) or Veh. (n = 20 for each group) and, 15 min later, they were placed on the hot plate device (51.5 ± 1°C). The time taken to elicit a flinching or jumping response (latency) was measured. The cut-off time was set at 45 s to minimize skin damage. In other sets of experiments, mice received a s.c. injection of pyrilamine or zolantidine or saline or Veh. and after 15 min, TA1 was injected i.c.v. (0.4, 1.32 and 4 $\mu g \cdot kg^{-1}$). Mice were then placed on the hot plate, 15 min after injection of TA1.

Nociceptive thresholds were evaluated in $HDC^{-/-}$ mice and $HDC^{+/+}$ mice, using the same procedures. Both strains of mice were injected i.c.v. with Veh or TA1 (1.32 and 4 $\mu g \cdot kg^{-1}$; n = 10 for each group of animals) and placed on the hot plate 15 min after TA1 injection.

Measurement of plasma glucose. Blood was collected from the tail veins of mice, which had been starved for 4 h, 15 min after i.c.v. injection of Veh or TA1 (0.4, 1.32 and 4 $\mu g \cdot kg^{-1}$, n = 10 mice for each treatment). Glucose concentrations in plasma were measured by a glucorefractometer, 15 min after TA1 injection.

Using the same procedures, plasma glucose was measured in mice pretreated with s.c. pyrilamine (10 $mg \cdot kg^{-1}$) or zolantidine (5 $mg \cdot kg^{-1}$) or saline, 15 min before receiving i.c.v. injection of TA1 (0.4, 1.32 or 4 $\mu g \cdot kg^{-1}$) or Veh (n = 10 for each group). Plasma glucose was also measured in $HDC^{-/-}$ and $HDC^{+/+}$ 15 min following i.c.v. injection of Veh. or TA1 (1.32 or 4 $\mu g \cdot kg^{-1}$; n = 10 for each group).

Data analysis

Data are expressed as mean ± SEM of independent experiments. Statistical analysis was performed by one-way ANOVA, followed by Student–Newman–Keuls multiple comparison *post hoc* test. When the experimental setting included only two groups, unpaired t-test was used. The threshold of statistical significance was set at $P < 0.05$. Data analysis was performed by GraphPad Prism 5.0 statistical program (GraphPad Software, San Diego, CA, USA).

Materials

TA1 was kindly provided by Dr Thomas Scanlan (Portland, OR, USA) and was dissolved in 0.5% DMSO (Veh). Pyrilamine, scopolamine and zolantidine were supplied by Sigma–Aldrich, St. Louis, MO.

Results

Endogenous TA1 in the brain of CD1 euthyroid mice

The brains of CD1 mice were analysed for TA1 and T1AM by HPLC coupled to mass spectrometry. Both T1AM and its oxidative derivative, TA1, were found in concentrations of 48.6 ± 17.7 and 0.8 ± 0.2 $pmol \cdot g^{-1}$ of tissue, corresponding to 17.3 ± 6.2 and 0.30 ± 0.06 $\mu g \cdot kg^{-1}$ (n = 3) respectively. So, the concentration of TA1 amounted to about 1.6% of that of T1AM.

TA1 modifies learning, reduces pain threshold and increases plasma glycaemia in CD1 mice

TA1, i.c.v. injected, at a dose close to its physiological levels (0.4 $\mu g \cdot kg^{-1}$) produced amnesia in 1 and 24 h retention sessions. On the contrary, at the doses of 1.32 and 4 $\mu g \cdot kg^{-1}$, mice remained in the light portion of the box for a significantly longer time in the 1 h retention session. Interestingly, and differently from what occurred with T1AM (Manni *et al.*,

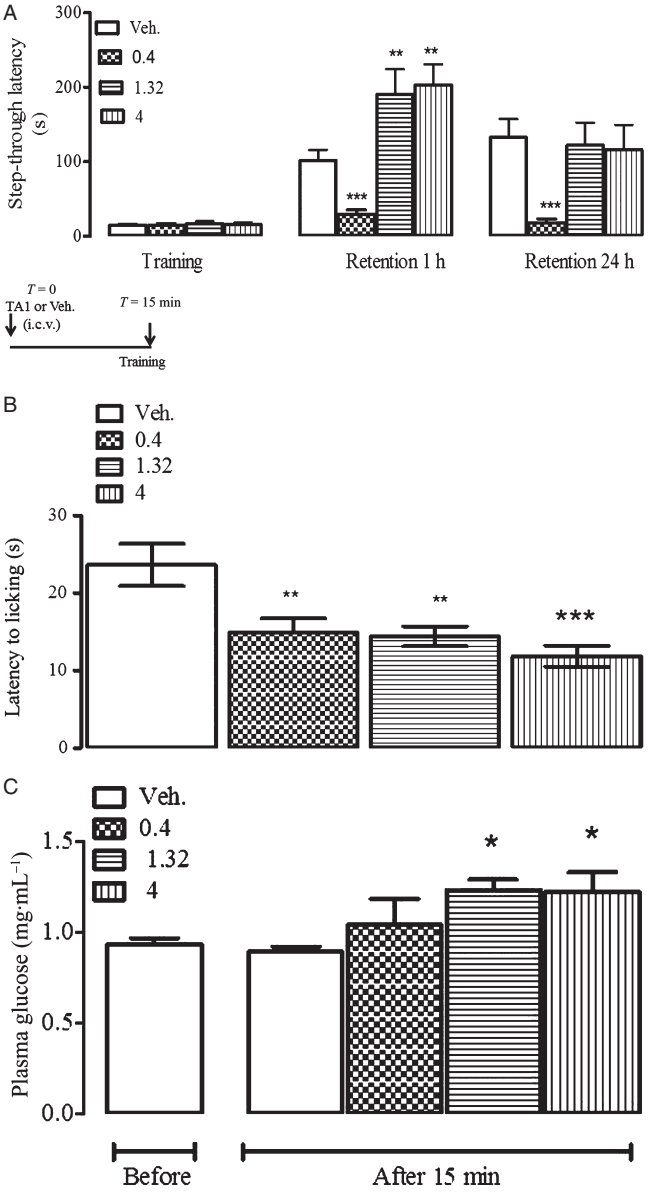

Figure 1

TA1 modifies learning, reduces nociceptive threshold and increases plasma glucose in mice. Mice (n = 20 for each groups of animals) were injected i.c.v. with TA1 (0.4, 1.32 or 4 μg·kg^{-1} or with Veh and, after 15 min, were assessed by the passive avoidance paradigm (A) or their nociceptive threshold measured by the the hot plate test (B). **P < 0.01, ***P < 0.001 versus Veh. Plasma glucose was also measured 15 min after TA1 injection, in blood collected from the tail veins of 4 h starved mice (n = 10 for r each groups of animals). Results are expressed as means ± SEM; *P < 0.05 versus Veh.

2013), memory consolidation was not observed in the 24 h retention session (Figure 1A).

In addition, 15 min after TA1 (0.4, 1.32 and 4 μg·kg^{-1}) injection, mice showed increased sensitivity (hyperalgesia) to thermal stimulus of the hot plate (Figure 1B), and at the doses of 1.32 and 4 μg·kg^{-1}, plasma glucose was higher than control values (Figure 1C).

Pretreatment with histamine receptor antagonists prevents stimulation of memory, hyperalgesia and increase of plasma glucose

TA1 was given i.c.v. to mice pretreated s.c. with saline or pyrilamine (10 mg·kg^{-1}), or zolantidine (5 mg·kg^{-1}), antagonists of histamine H_1 and H_2 receptors respectively. As shown in Figure 2A–C, pretreatment with the histamine receptor antagonists *per se* did not modify memory acquisition, nociceptive threshold or plasma glucose , but it markedly affected the behavioural responses induced by TA1.

Pyrilamine pretreatment abolished the pro-learning effect of 1.32 and 4 μg·kg^{-1} TA1, while the amnesic effect of 0.4 μg·kg^{-1} TA1 was maintained (Figure 3A). In mice pretreated with zolantidine, TA1, induced amnesia at all the doses studied (Figure 3A).

Pretreatment with pyrilamine or zolantidine also affected the hyperalgesic and the hyperglycaemic effect of TA1. In particular, after either the H_1 or H_2 receptor antagonists, hyperalgesia was induced only by the dose of 4 μg·kg^{-1} (Figure 2B) and plasma glucose did not rise at any of the doses injected (Figure 2C). Taken together, these results suggested that stimulation of memory, hyperalgesia and increase of plasma glucose might have a common mechanism involving the histaminergic system.

TA1, at the dose stimulating learning, was also anti-amnesic

Memory acquisition and retention is a complex behaviour controlled by an organized network of integrated signalling including the muscarinic receptors. Because of this, scopolamine injection in rodents is often used to provide an experimental model of amnesia and we tested the effects of TA1 on such a model. In mice pretreated i.p. with scopolamine, at a dose (0.3 mg·kg^{-1}) which produced amnesia (Rush, 1988) without inducing analgesia or sedative effects, injection of 1.32, but not 4 μg·kg^{-1}, TA1 completely reversed the amnesia, giving consolidation in the 1 h and also in the 24 h retention session (Figure 3).

T1AM and TA1 in the brain of HDC$^{+/+}$and HDC$^{-/-}$ mice

The rate-limiting step in the biosynthesis of histamine is catalysed by HDC. Although there are inhibitors of HDC enzymic activity, genetic silencing of the HDC gene represents a strategy to obtain a mouse lacking histamine. Because of this, HDC$^{-/-}$ mice could provide a suitable model to assess the participation of the histaminergic system in the effects of TA1. Interestingly, the WT (HDC$^{+/+}$) mice exhibited significantly lower brain levels of T1AM (0.22 ± 0.03 pmol·g^{-1} of tissue; n = 4, P < 0.001) than found in CD1 mice (about 48 pmol·g^{-1} of tissue, see above) and the levels of TA1 were below the limit of detection. In HDC$^{-/-}$ mice,.neither T1AM nor TA1 could be detected in brain tissue.

T1A effects in HDC$^{-/-}$ mice

During the training for the passive avoidance paradigm, only a very few HDC$^{+/+}$ or HDC$^{-/-}$ animals (1/15) entered the dark side. This pattern of behaviour did not permit the effects of TA1 on this task to be evaluated. However, both HDC$^{+/+}$

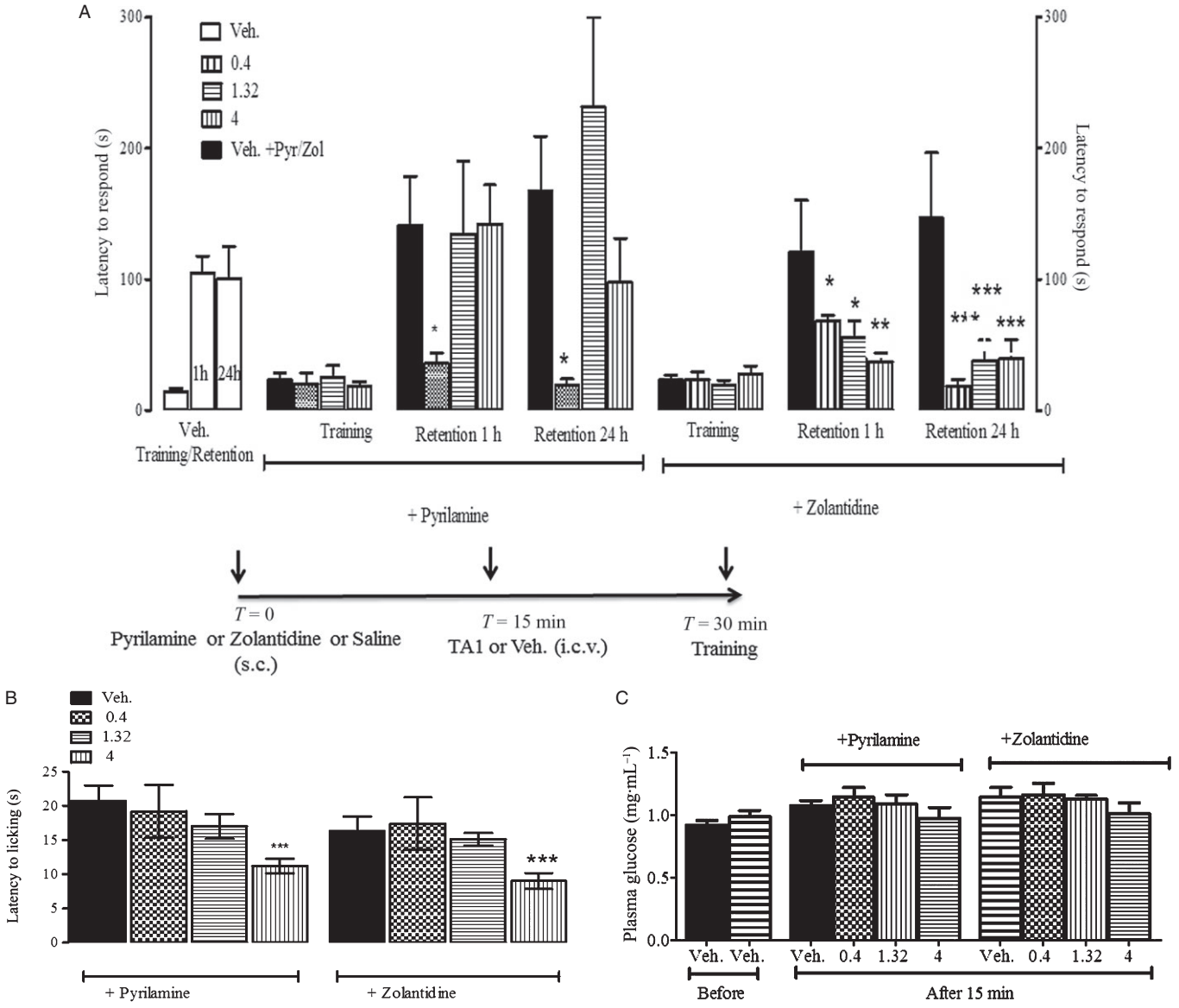

Figure 2

The effects of histamine H_1 and H_2 receptor antagonists on TA1-induced modification of learning, pain threshold and plasma glucose in mice. Mice (n = 20 for each groups of animals) were pretreated s.c. with a single injection of pyrilamine (10 mg·kg^{-1}) or zolantidine [5 mg·kg^{-1}, 15 min before the i.c.v. injection of T1A (0.4, 1.32 and 4 μg·kg^{-1}) or Veh.]. 15 min after TA1 or Veh injection, mice were assessed by the passive avoidance paradigm (A), or nociceptive thresholds determined by the hot plate test (B). Plasma glucose was also measured 15 min after TA1 (0.4, 1.32 and 4 μg·kg^{-1}) i.c.v. injection in the blood collected from the tail veins of 4 h starved mice (n = 10 for each treatment) (C). Results are expressed as means ± SEM. *P < 0.05, **P < 0.01,***P < 0.001 versus Veh.

and HDC$^{-/-}$ mice were responders in the hot plate test. In HDC$^{+/+}$ mice, the i.c.v. injection of TA1 (1.32 and 4 μg·kg^{-1}) produced hyperalgesia (Figure 4A) and raised plasma glucose (Figure 4B). On the contrary, in HDC$^{-/-}$ mice, neither a hyperalgesia nor increased plasma glucose was observed following TA1 injection (Figure 4C and 4D)

Discussion

We here provide, for the first time, evidence that endogenous TA1 was found in the brain of CD1 mice, representing 1.6% of its putative precursor T1AM. In these animals, exogenous TA1, injected i.c.v., produced rapid (within 15 min) effects including modification of memory, of nociceptive threshold and of plasma glucose. All these effects were modulated by histamine H_1 and H_2 receptor antagonists. Furthermore, TA1 failed to raise plasma glucose and to induce hyperalgesia when injected in mice lacking HDC, indicating that these effects of TA1 (1.32 and 4 μg·kg^{-1}) were mediated by histamine release.

TA1 has been identified as the main oxidative metabolite of T1AM in cardiac (Saba *et al.*, 2010) and thyroid cells (Agretti *et al.*, 2011). Moreover, TA1 was recovered in mouse

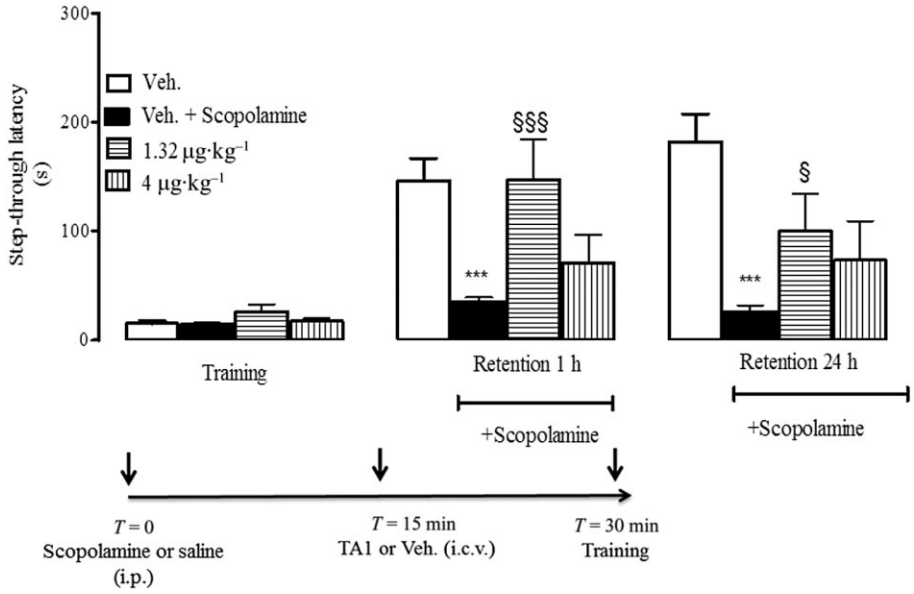

Figure 3

TA1 reversed scopolamine-induced amnesia. TA1, 1.32 and 4 µg·kg^{-1} (n = 10 for each dose) or Veh were injected i.c.v. in mice (n = 20 for each groups of animals) pretreated i.p. with scopolamine (0.3 mg·kg^{-1}, n = 30) or saline. Mice were then assessed by the passive avoidance paradigm. Results are expressed as means ± SEM. §P < 0.05 and §§§P < 0.001 versus Veh. + scopolamine, ***P < 0.001 versus Veh.

serum following i.p. administration of T1AM (Hackenmueller and Scanlan, 2012), supporting the concept that T1AM and TA1 are endogenous derivatives of the thyroid hormone and that the latter can be produced from the former. Before the present data, the evidence for TA1 in mouse brain was lacking. In this respect, our data demonstrated the presence of TA1 and of T1AM, the amine from which it derives, in CD1 mice and also provide an estimate of their reciprocal levels. Furthermore, our data indicating very low levels of T1AM or TA1 in mice of the 129/Sv strain (HDC$^{-/-}$ or HDC+/+), suggest there is a considerable strain-related difference in thyroid hormone metabolism.

One way to demonstrate the function of endogenous compounds is to observe the effects produced by their pharmacological administration in rodents. Here, we have demonstrated that i.c.v. injection of TA1, at doses close to its endogenous levels, modified behaviour, including memory acquisition and reduced nociceptive thresholds and raised plasma glucose. In particular, the effect of TA1 on memory was dose-dependent, ,with the lowest dose at 0.4 µg·kg^{-1} exerting amnesic effects but being pro-learning at 1.32 and 4 µg·kg^{-1} without retention in the 24 h post-training session. Overall, these results show a difference between TA1 and T1AM, as the latter when used in almost equimolar doses, to those used in this paper for TA1, never produced amnesia (Manni *et al.*, 2012). Given that that very little is known of the pharmacokinetics of TA1 and of the impact, if any, of exogenous TA1 on endogenous levels of T1AM, one can hypothesise that the acid induces the release amnesic or pro-learning mediators depending on its levels at cell target(s), or that T1AM effects are not entirely mediated by TA1.

However, at all the doses tested, TA1, like T1AM, induced hyperalgesia and hyperglycaemia. All these findings indicated TA1, like T1AM, had the potential to behave as a novel mediator, and encouraged elucidation of its mechanism of action.

We first observed that the effects of TA1 (stimulation of memory, reduction of pain threshold and increase of plasma glycaemia) were not modulated by amiloride (40 µmol·kg^{-1} i.p.; data not shown), a non-selective antagonist of the acid-sensitive channels involved in memory and pain (Chu *et al.*, 2011). Modification of memory, increase of plasma glucose and reduction of pain threshold were reported following i.c.v. injection of histamine and of selective histamine receptor agonists in rodents (Nishibori *et al.*, 1987; Ishibory *et al.*, 1990; Galeotti *et al.*, 2004). Because of this, we explored the hypothesis that histamine might be involved in the effects of TA1. Our data showed that in the presence of H_1 or H_2 receptor antagonists, the pro-learning, the hyperalgesic and the hyperglycaemic effects induced by TA1 were all markedly changed, suggesting the involvement of histamine in all these effects of TA1.

It is well known that the effects of histamine and related compounds on memory depend on the brain region where they are injected, on the type of test used and on the receptor subtype involved (Köhler *et al.*, 2011), complicating the interpretation of results. For instance, drugs modulating the histamine H_3 receptor reverse scopolamine-induced amnesia in different experimental settings (Köhler *et al.*, 2011). We found, here, that T1A (1.32 but not 4 µg·kg^{-1}) reversed scopolamine-induced amnesia, producing a long-lasting memory consolidation, which persisted in the 24 h retention session. This latter result potentially suggests H_3 receptors as a target for TA1, a hypothesis, which, however, needs appropriate experiments to be verified. In any case, this anti-amnesic effect adds to our knowledge of the effects of TA1 on memory and may further support the involvement of histamine release in the pharmacological effects of TA1.

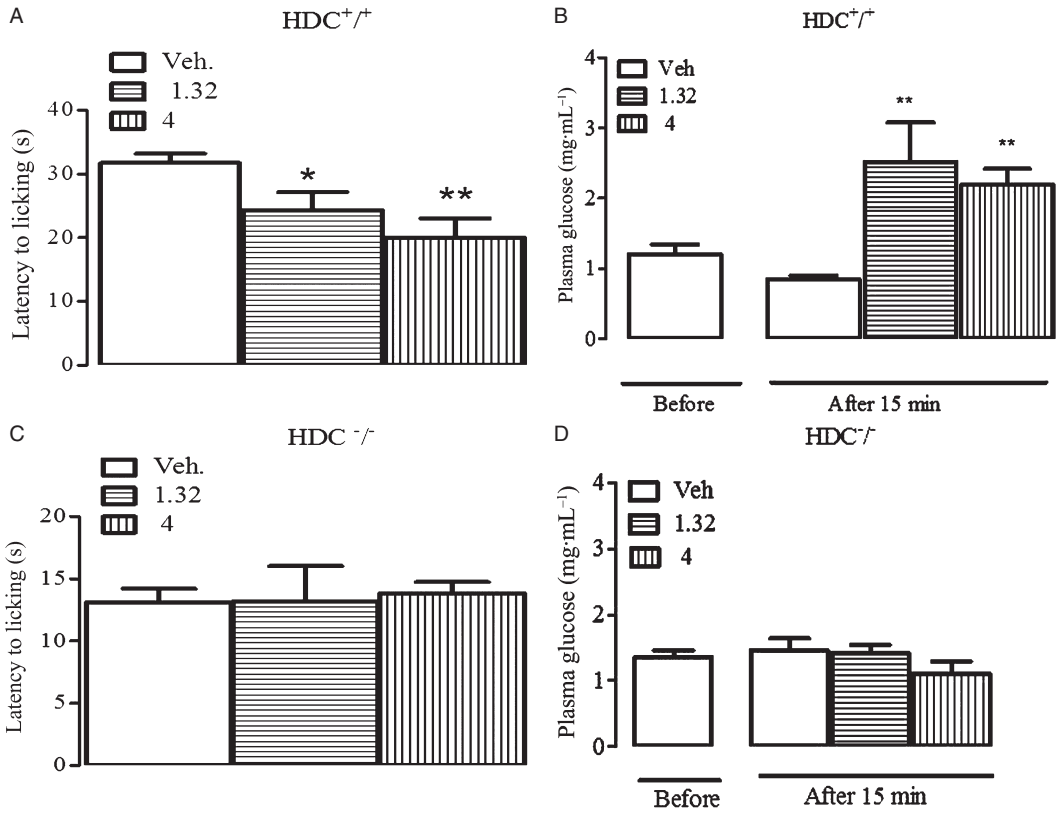

Figure 4

T1A failed to induce hyperglycaemia and to raise plasma glucose when injected i.c.v. in HDC$^{-/-}$ mice. TA1 (1.32 and 4 μg·kg^{-1}), or Veh, were injected i.c.v. in HDC$^{+/+}$ and HDC$^{-/-}$ mice ($n = 10$ for each group of animals). After 15 min, nociceptive thresholds to a thermal stimulus and plasma glucose were evaluated in HDC$^{+/+}$ (A and B) and in HDC$^{-/-}$ mice (C and D). Results are presented as the means ± SEM. *$P < 0.05$ versus Veh; **$P < 0.01$ versus Veh.

In our experimentals, it is not possible to locate the sites where histamine release could have occurred as i.c.v. injection of TA1 ensures that it was distributed throughout the CNS. However, we observed that pretreatment of mice with pyrilamine abolished the pro-learning effect of 1.32 and 4 μg·kg^{-1} TA1, whereas the amnesic effect of 0.4 μg·kg^{-1} TA1 was maintained. In contrast, with zolantidine pretreatment, TA1 was amnesic at all the doses tested. As we used a single antagonist in our experiments , we cannot draw conclusions on the receptor subtype involved in these effects. On the other hand, treatment with more than one antagonist could produce ambiguous results. With all the limitations of the settings, a major involvement of histamine H_2 and H_1 receptors in the pro-learning and amnesic effects, respectively, of TA1 can be postulated.

Pyrilamine and zolantidine pretreatment also affected hyperalgesia and hyperglycaemia induced by TA1. In particular, in the presence of H_1 or H_2 receptor blockade, the hyperalgesic and hyperglycaemic effects of TA1 were evident only at the highest dose of 4 μg·kg^{-1}, a finding, which again cannot exclude the involvement of the H_3 receptor subtype (Hough and Rice, 2011).

Evidence indicating that the hyperalgesic and hyperglycaemic effects of TA1 were linked to histamine release were obtained in the HDC$^{-/-}$ mice. In this animal model, TA1 injection (1.32 and 4 μg·kg^{-1}) failed to increase plasma glucose and to induce hyperalgesia to the thermal stimulus. Instead, in HDC$^{+/+}$ mice both hyperalgesia and hyperglycaemia were evident with the latter effect being greater than in CD1 mice. Unfortunately, we could not verify the effect of TA1 on learning behaviour in HDC$^{+/+}$ and HDC$^{-/-}$ mice because, as described in the Results, both these strains failed in the passive avoidance paradigm. The reason for such a failure is not known at present, but it may be related to the absence of TA1 in their brains. In fact, our results showed that in both HDC$^{+/+}$ and HDC$^{-/-}$ brain levels of TA1 were undetectable, indicating that there is a marked strain-dependent difference in endogenous levels of this metabolite of the thyroid hormone. However, taken together, our results strengthened the interaction between histamine and metabolites of the thyroid hormone.

Based on the neurological signs and symptoms associated with thyroid disorders in adults, the thyroid hormone plays fundamental roles in the nervous system, well after the well-known effects on development are complete. As an animal matures, subcellular accumulation of thyroid hormone in neurons changes to a characteristically adult pattern of concentration suggesting non-genomic, neurotransmitter-like mechanisms of action (Dratman and Gordon, 1996). In line with this hypothesis, our findings confirm that an

end-product of T3 metabolism, TA1, may be considered a novel endogenous mediator, active in memory and nociceptive sensitivity, two physiological processes known to be modified in conditions of thyroid dysfunction (Guieu *et al.*, 1993; Rivas and Naranjo, 2007). As TA1 is generated by oxidative deamination (by constitutive MAO activity) and/or deiodination of amine derivatives of thyroid hormone metabolites, the intracellular bioavailability of T3 potentially represents the limiting step in TA1 formation. Furthermore, endogenous levels were TA1 are strain-specific and would be expected to be modified under conditions of overexpression or pharmacological inhibition of the enzymes involved in its biosynthesis (Pino *et al.*, 1997; Metszaros *et al.*, 1999; Masini-Repiso *et al.*, 2004; Petrovic *et al.*, 2005). The consequences of each of these circumstances on the pharmacological effects of TA1 are not known and represent a potential topic of research interest.

Thyromimetic compounds devoid of genomic activity have been extensively studied for their potential therapeutic usefulness in ameliorating hyperlipidaemia and the associated cardiovascular risk (Tancevski *et al.*, 2011). We here present TA1 as a novel endogenous thyromimetic whose (non-genomic) behavioural and metabolic effects reveal the existence of a signalling pathway integrating thyroid and the histaminergic system. This interaction might have clinical relevance in explaining the occurrence of some symptoms associated with thyroid dysfunctions (Hiramanek, 2004).

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Conflict of interest

The authors declare no competing financial interests.

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