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EFFECT OF THYROTROPIN RELEASING HORMONE AND SOME OF ITS HISTIDINE ANALOGS ON THE CARDIOVASCULAR SYSTEM AND PROLACTIN RELEASE IN THE CONSCIOUS RAT

A.-L. Siren, G. Feuerstein, V.M. Labroo[2], L.A. Cohen[2], and D. Lozovsky[1]

Neurobiology Research Division, Department of Neurology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799, [1]Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20205 and [2]National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205 (reprint requests to ALS).

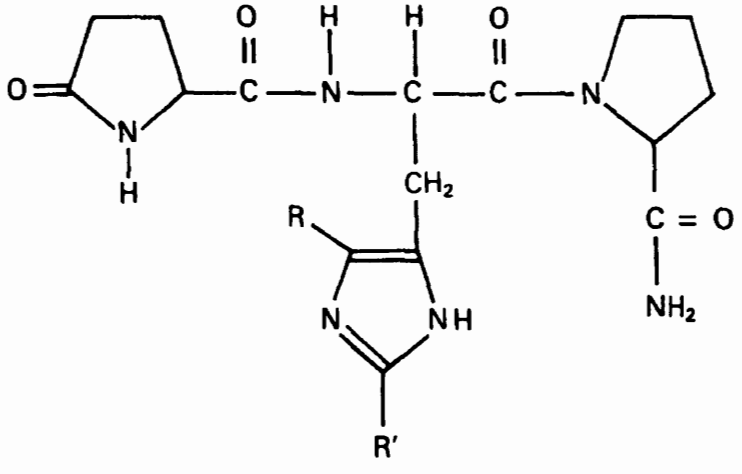
ABSTRACT

The cardiovascular and endocrine activity of three analogs of thyrotropin releasing hormone (TRH), 4-nitro-imidazole TRH (4-nitro-TRH), 2-trifluoro-methyl-imidazole TRH (2-TFM-TRH) and 4-trifluoro-methyl-imidazole TRH (4-TFM-TRH), was compared to TRH in conscious rats. Injection of TRH or the three analogs (1 mg/kg or 5 mg/kg) into the arterial line induced increases in mean arterial pressure, pulse pressure and heart rate and raised plasma prolactin (PRL). None of the analogs were more potent than TRH in inducing cardiovascular changes. The 4-TFM-TRH was significantly less potent than the 2-TFM-TRH in increasing blood pressure, while the nitro-TRH was more potent than the 2-TFM-TRH in producing tachycardia. TRH induced a two-fold increase in PRL at the 5 mg/kg dose, while both the fluorinated analogs elicited a 4 to 5 fold increase in PRL at the higher dose. The present results suggest that the receptors for TRH-elicited PRL release differ from TRH-receptors involved in its cardiovascular actions.

INTRODUCTION

In addition to the thyrotropin release, thyrotropin releasing hormone (TRH) releases prolactin (PRL) from the pituitary of several mammalian species (1). Moreover, TRH has central nervous system actions which are totally unrelated to its effect on the hypothalamo-pituitary axis (2,3). In both experimental animals (4-7) and humans (8,9), TRH produces pressor and tachycardic responses. The variety of autonomic endocrine and neural functions regulated by TRH suggests that different receptors might mediate the various effects of TRH. The existance of multiple TRH receptors is supported by both biological (10,11) and biochemical studies (3,12,13). Appropriate modification of the TRH molecule might, therefore, yield analogs with an action selective for either neuroendocrine or central nervous system.

Previous studies have shown that modification of the imidazole ring of histidine has profound effects on the biological activity of TRH. The N-methylated analog, 3-Me-Im-TRH, is about ten times as active as TRH in assays for TSH release while the 1-methyl analog is inactive (14). Replacement of histidine by [Pyr(1)Ala^2] also gives an active analog while [Pyr(3)Ala^2] is only weakly active (15). Our previous studies have shown that 4-F-Im-TRH (4-Fluoro-TRH) and 2-TFM-TRH fail to bind to GH_4 cells or release prolactin from them (16). On the other hand, discrete central injection of these analogs to rats results in cardiovascular actions and PRL responses somewhat similar to those of TRH (17). In order to elaborate the effect of imidazole-ring substitution in TRH on its biological profile, we have continued to synthetize and evaluate analogs in which the imidazole ring is modified by electron-releasing or electron-withdrawing groups in the 2- and/or 4-position. As part of this study, we have prepared 2-trifluoromethyl-Im-TRH (2-TFM-TRH), 4-trifluoromethyl-Im-TRH (4-TFM-TRH) and 4-nitro-Im-TRH (nitro-TRH) (18, Fig. 1) and report here the effects of these analogs on blood pressure, heart rate and plasma PRL levels in conscious rats.



TRH	R = R′ = H
4-CF_3-TRH	R = CF_3, R′ = H
2-CF_3-TRH	R = H, R′ = CF_3
4-NO_2-TRH	R = NO_2, R′ = H
4-F-TRH	R = F, R′ = H

Figure 1. Chemical structure of TRH, 2-TFM-TRH, 4-TFM-TRH and nitro-TRH.

MATERIAL AND METHODS

Male Sprague-Dawley rats (250-300 g) were purchased from Taconic Farms (Germantown, NY) and kept at 22°C and 12hr/12hr light/dark cycle. Polyethylene catheters (PE-50) were inserted into the femoral arteries under halothane (2% in oxygen) anesthesia. The catheters were tunneled under the back skin and exteriorized at the nape of the neck. The lines were secured by a soft spring wire throughtout the cage as earlier described (19). Twenty-four hours after the surgery the arterial line was attached to a pressure transducer (Narco RP 1500i) and continuous recordings of blood pressure (systolic, diastolic, mean) and heart rate were carried out by the Narcotrace 80 computerized physiograph. In addition, arterial blood sample (0.5 ml) was withdrawn before drug injection and at 5 and 30 min after the injection. Each blood sample withdrawn was replaced by an equal amount of fresh rat blood. This is a standard procedure in our conscious, chronically instrumented rats.

Assay of plasma prolactin

Plasma PRL levels were determined by radioimmunoassay using antibodies and standard (RP-2) supplied by the National Institute of Arthritis Metabolism and Digestive Diseases (20). This antibody was used in final assay dilution of 1:20 000. Iodinated PRL was obtained from New England Nuclear (Boston, MA;SA 20-5 Ci/μg), and 10 000 cpm/tube were used. Following a 24 hr incubation at 24°C, bound material was separated using polyethylene glycol (21). The sensitivity of the assay is 25pg/ml.

Drugs used

Thyrotropin releasing hormone (Sigma Chemical Co.) and the NO_2-, 2-TFM- and 4-TFM analogs (Fig 1) were dissolved in saline (0.9% NaCl) and injected into the arterial line at the doses of 1mg/kg and 5mg/kg.

Data in text and figures are mean±SEM for the indicated number of rats. Analysis of variance followed by the Student-Newman-Keul test for multiple comparisons was used for statistical evaluation of the data.

RESULTS

Effect of the TRH analogs on blood pressure and heart rate

The effects of TRH and the three analogs on mean arterial pressure, pulse pressure and heart rate are shown in Figure 2. Injection of TRH or the three analogs (1 or 5mg/kg) into the arterial line increased the blood pressure and heart rate. The maximum increments of mean arterial pressure and heart rate were reached 5-15 min after each injection and the effect subsided in 30-45 minutes. None of the analogs tested were more potent than TRH in eliciting increases in mean arterial pressure, pulse pressure or heart rate. The 4-TFM-TRH was significantly less potent than the 2-TFM-TRH in increasing blood pressure, while the nitro-TRH was significantly more potent than the

2-TFM-TRH in producing tachycardia. The increase in pulse pressure by the 4-TFM-TRH was also significantly lower than the effect induced by native TRH.

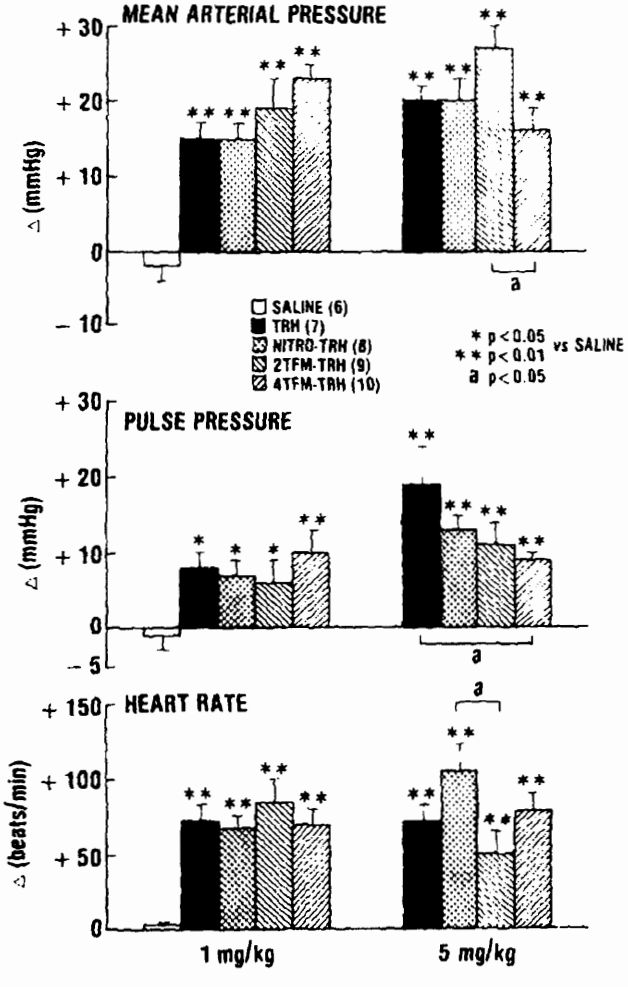


Figure 2. Maximum increases in mean arterial pressure, pulse pressure and heart rate following intra-arterial administrations of saline TRH, 2-TFM-TRH, 4-TRM-TRH and nitro-TRH in the conscious rat. Number of rats in each group is given in parenthesis. Vertical bars indicate S.E.M. Asterisks and "a" denote statistical significance by Student-Newman-Keul test.

Effect on plasma prolactin

The effect of TRH and the analogs on plasma PRL is demonstrated in the Figure 3. The higher dose of TRH (5mg/kg) induced a twice-fold increase in plasma PRL. The maximum effect was achieved within 5 min after the injection and the levels were back to baseline values 30 min after the injection. Both the fluorinated analogs induced a 4-5 fold increment in plasma PRL at the 5mg/kg dose, and the 2-TFM-TRH significantly raised the PRL levels even at the lower dose. The nitro-TRH elicited a significant decrease in the plasma PRL with the 1mg/kg dose but had no effect at the higher dose. The effect of the nitro-TRH on PRL at the 1 mg/kg dose was signifcantly attenuated as compared to the increases in PRL induced by TRH, 2-TFM-TRH or 4-TFM-TRH.

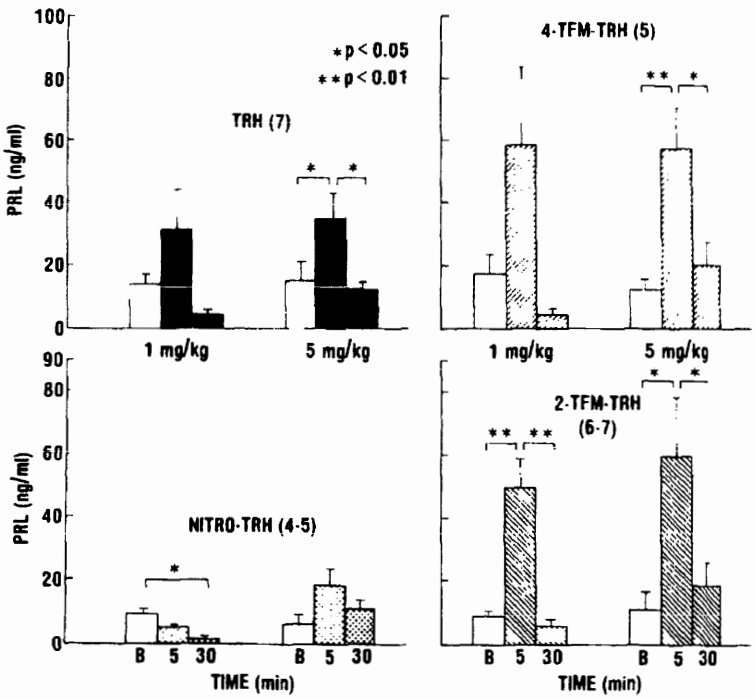


Figure 3. Effects of TRH, 2-TFM-TRH, 4-TFM-TRH and nitro-TRH on plasma prolactin (PRL) level in the conscious rat. The two doses of each drug (1mg/kg and 5mg/kg) were injected into the arterial line 120 min apart from each other. Baseline level of PRL is denoted by "B", 5 and 30 indicates the appropriate time points after the drug administrations. Numbeer of rats in each group is given in parenthesis. Vertical bars indicate S.E.M. Asterisks denote statistical significance from baseline levels by Student-Newman-Keul test. The difference in baseline levels of PRL between the groups was not statistically significant. The increase in PRL induced by the nitro-TRH 5 min after the 1mg/kg dose was dignificantly attenuated as compared to the increment of PRL produced by TRH, 2-TFM-TRH or 4-TRM-TRH (0<0.01).

DISCUSSION

The present results confirm the previous findings that TRH and its imidazole substituted analogs induce increments of blood pressure and heart rate and release prolactin (17). All the three analogs used in the present study were equipotent to TRH in eliciting cardiovascular changes. On the other hand, the 4-nitro-TRH was far less potent than the native TRH in inducing prolactin release, while the 2-TFM-TRH and 4-TFM-TRH-analogs were significantly more potent than TRH in increasing plasma PRL. In a previous study (17) it has been reported that TRH and its 4-fluoro analog produced increases in blood pressure and heart rate and released prolactin also upon microinjections of nanomoles (28 nmol=10 μg) of these drugs into the hypothalamic nucleus preopticus medialis (POM). The 2-TFM-TRH was significantly less potent than TRH or the 4-fluoro-TRH in inducing tachycardia, while we did not find any significant differences between the cardiovascular effects of TRH and the 2-TFM-TRH. The difference in the route of administration might explain this difference, i.e., systemic administration of the compound might gain access to multiple brain sites in which cardiovascular responses can be elicited. The cardiovascular effects of TRH in the conscious rat are, however, similar both upon POM (6), intracerebroventricular (22) and systemic administrations (23).

The biological effects of 4-nitro-TRH and of 4-TFM-TRH have not been described previously. Although both analogs bear strong electronegative substituents at C-4 of the imidazole ring, their biological profiles are quite different. While they are equipotent with TRH in inducing cardiovascular changes, their effects on plasma PRL release stand in marked contrast. 4-Nitro-TRH is almost devoid of PRL activity while 4-TFM-TRH is significantly more active than TRH. These data therefore strongly suggest that the TRH receptors which mediate PRL release are different from the receptors involved in the cardiovascular effects of TRH. This selectivity might be due to the difference in substitutions in the imidazole ring of TRH analogs. The nitro group is not only more electronegative than trifluoromethyl group but is much more capable of participating in intramolecular (or intermolecular) hydrogen bonds. Either or both of these effects may be directly responsible for selective binding to different TRH receptors, or indirectly responsible by creating different conformations of the peptide. Indeed, high resolution proton NMR spectroscopy does suggest conformational differences in the analogs by virtue of differences in coupling constant between the α-CH and β-CH_2 groups of histidine. Detailed NMR analysis of backbone and side chain conformations of these peptides is under investigation.

It is now evident, therefore, that differentiation in the biological activities of TRH can be achieved by selective substitution in the imidazole ring of histidine. Thus, trifluoromethylated analogs of TRH may prove preferable to TRH in clinical assays based on prolactin release, while 4-nitro-TRH may be useful in clarifying the role of TRH in regulation of the cardiovascular system or in the treatment of shock states.

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