NEUROPEPTIDES

Neuropeptides (1987) 10, 29–36

DISSOCIATION OF THE CARDIOVASCULAR AND PROLACTIN-RELEASING ACTIVITIES OF NORVALINE^2-TRH

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ABSTRACT

The effects of thyrotropin-releasing hormone (TRH) and norvaline^2-TRH (Nva^2-TRH) on blood pressure, heart rate and plasma prolactin levels in conscious rats have been compared. Systemic injection of TRH or Nva^2-TRH (1 mg/kg or 5 mg/kg) produced equipotent increases in plasma prolactin. On the other hand, while TRH significantly increases blood pressure and heart rate, Nva^2-TRH was essentially inactive. Thus, two contrasting analogues are now available: 4-NO_2-Im-TRH (Neuropeptides, 8, 63, 1986) has full cardiovascular activity and no PRL-releasing activity, while Nva^2-TRH has no cardiovascular activity and full PRL-releasing activity of TRH.

INTRODUCTION

Thyrotropin-releasing hormone (TRH) is known to stimulate the release of both thyrotropin and prolactin (PRL) from the pituitary of mammalian species (1). TRH has also been shown to have a direct effect on the central nervous system (CNS), which may be related to its antidepressant activity and unrelated to its effect on the hypothalamo-pituitary axis (2,3). Simultaneously, TRH produces pronounced pressor and tachycardiac effects in both experimental animals (4-7) and humans (8,9), probably through activation of the sympatho-adrenomedullary system (6).

Various TRH analogues have been shown to possess increased CNS effects and reduced thyrotropin-releasing activity (10). This selectivity has been achieved by modifying either the proline ring (pGlu-His-Tzl-NH_2), the pyrrolidone ring (pAad-His-Pro-NH_2) or both (pAad-His-Tzl-NH_2); the doubly modified peptide is 35 times more active than TRH on CNS functions and has only 1/5 the activity of TRH in releasing thyrotropin. Other modifications of proline (pGlu-His-DmPro) also lead to increased CNS activity (11,12). These changes in activity are apparently due to increased metabolic stability and to greater ability

to cross the blood brain barrier (12,13). Modifications of the imidazole ring of histidine have also been shown to have a profound effect on the thyrotropin-releasing activity of TRH (14). We have previously shown that introduction of fluorine (4-F-Im-TRH) or of the trifluoromethyl group (4-CF_3-Im-TRH and 2-CF_3-Im-TRH) into the imidazole ring of TRH results in analogues which show both the cardiovascular and PRL-releasing effects of TRH (15-18); however, the trifluoromethyl analogues are 2-3 times more active than TRH in releasing PRL.

Introduction of a stronger electron-withdrawing group (as in 4-NO_2-Im-TRH) totally abolishes PRL-releasing activity but retains cardiovascular responses equipotent with that of TRH (18); thus, the two activities can be totally dissociated. Recently, Nva^2-TRH, in which histidine has been replaced by norvaline, was shown to have a CNS (analeptic) effect but no thyrotropin-releasing activity (19). This is the first example of total dissociation of CNS and pituitary activities. However, the effect of Nva^2-TRH on cardiovascular or other neuroendocrine function (e.g., prolactin release) were not studied.

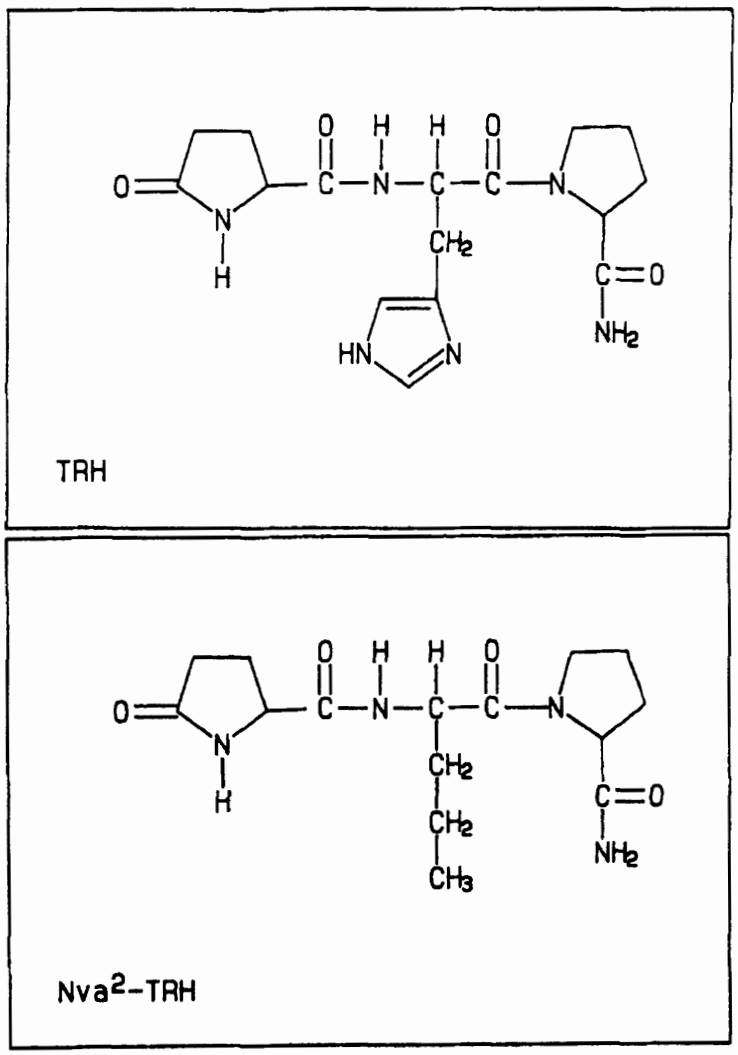

Figure 1. Chemical structures of TRH and Nva^2-TRH

In further pursuit of our efforts to dissociate pituitary from cardiovascular activities, we have studied the effects of TRH and Nva^2-TRH (Figure 1) on blood pressure, heart rate and plasma PRL levels in conscious rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats (250-300g) were purchased from Taconic Farms (Germontown, 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 throughout the cage as earlier described (20). Twenty-four hours after the surgery, the arterial line was attached to a pressure transducer (Narco RP 1500i). Blood pressure (systolic, diastolic, mean), and heart rate were continuously recorded by Narcotrace 80 computerized physiograph. In addition, arterial blood samples (0.5ml) were 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 Diabetes and Digestive and Kidney Diseases (21). This antibody was used in final assay dilution of 1:20,000. Iodinated PRL was obtained from New England Nuclear (Boston, MA; SA 20-25 Ci/μg), and 10,000 cpm/tube were used. Following a 24hr incubation at 24°C, bound material was separated using polyethylene glycol (22). The sensitivity of the assay is 25 pg/ml.

Drugs used

Thyrotropin-releasing hormone was purchased from Sigma Chemical Co. Norvaline^2-TRH (Figure 1) was synthesized by solution phase peptide synthesis. The drugs 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 $\pm$ 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. A p value of $p<0.05$ was considered of significant difference.

RESULTS

Effect of TRH and Nva^2-TRH on blood pressure and heart rate

The effects of TRH and Nva^2-TRH (1 or 5 mg/kg) on mean arterial pressure (MAP), pulse pressure (PP) and heart rate (HR) are shown in Figure 2. Injection of TRH into the arterial line increased blood pressure and heart rate, with a more striking effect at the higher dosage. The maximum increases in MAP and HR were reached within 5-15 min after injection and the effect subsided in 30-45 min. Nva^2-TRH, injected in similar fashion, did not show any significant increases in blood pressure or heart rate at either dosage.

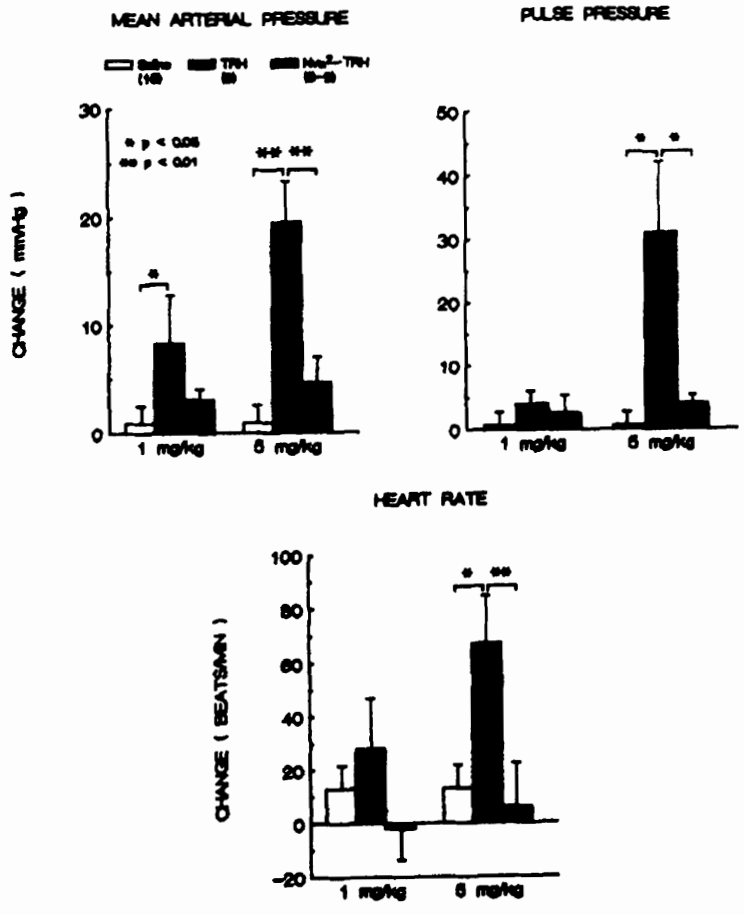

Figure 2. Maximum increases in mean arterial pressure, pulse pressure and heart rate following intra-arterial administrations of saline, TRH and Nva^2-TRH in conscious rats. Number of rats in each group is given in parentheses. Vertical bars indicate S.E.M. Asterisks denote statistical significance by Student-Newman-Keul test.

Effects on plasma prolactin

The effects of TRH and Nva^2-TRH (1 or 5 mg/kg) on plasma prolactin levels are shown in Figure 3. The compounds were almost equipotent in inducing increases in plasma PRL at either dosage, although the 5 mg/kg dose appeared to be no more effective than 1 mg/kg. The maximum effect was achieved within 5 min after injection and the levels had returned essentially to baseline about 30 min after injection.

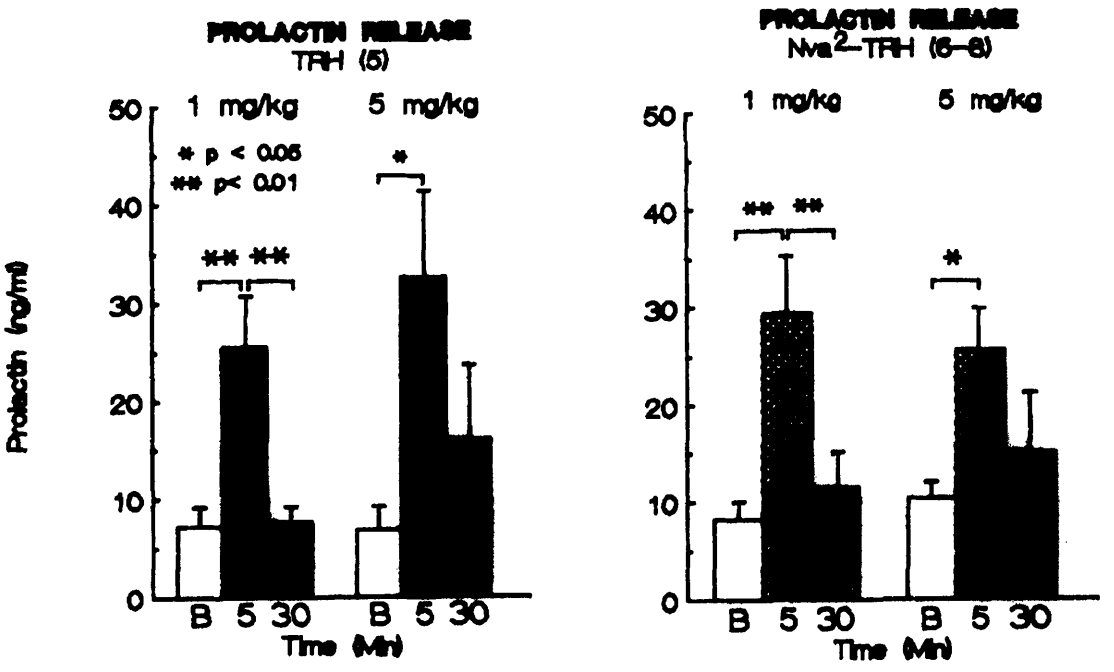

Figure 3. Effects of TRH and Nva^2-TRH on plasma prolactin (PRL) levels in conscious rats. The two doses of each drug (1 mg/kg and 5 mg/kg) were injected into the arterial line 120 min apart from each other. Baseline levels of PRL are denoted by "B"; 5 and 30 indicate the appropriate time (in min) points after the drug administrations. The number 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.

DISCUSSION

The present studies show that replacement of histidine in TRH by a hydrophobic amino acid, such as norvaline, serves to abolish the cardiovascular activity of TRH but to retain the ability to release prolactin. Since Nva^2-TRH has been reported to be devoid of thyrotropin-releasing activity (19), it would appear either that, PRL release and thyrotropin release are controlled by different pituitary receptors, or that PRL release is regulated both by pituitary and nonpituitary receptors. Although cardiovascular effects of TRH are mediated through the CNS (23), Nva^2-TRH shows CNS (analeptic) activity (19) but no cardiovascular activity. Here again, the evidence points to the involvement of different receptors for the two central effects.

We have previously shown that various modifications of the imidazole ring of histidine in TRH provide analogues with cardiovascular effects similar to those of TRH. Thus, 4-F-Im-TRH, 4-CF_3-Im-TRH, 2-CF_3-Im-TRH and 4-NO_2-Im-TRH induce increases in blood pressure and heart rate similar to those of TRH (15-18). It seems, therefore, that the imidazole ring, perhaps by virtue of its hydrogen-bonding ability, may be essential for the cardiovascular activity. On the other hand, this feature does not appear to be critical for the PRL-releasing activity of TRH.

Our studies have provided so far two analogues which offer striking contrasts: 4-NO_2-Im-TRH has full cardiovascular activity and no PRL-releasing activity; on the other hand, Nva^2-TRH has no cardiovascular activity and full PRL-releasing activity. The former analogue may be useful in elucidating the role of TRH in the regulation of cardiovascular system or in the treatment of hypotensive states or various forms of shock (24,25); the latter should be preferable to TRH in clinical assays based on prolactin release. We are, therefore, encouraged to continue our efforts to create additional analogues, each selective for only one of the multiple TRH receptors, to furnish selective agonists for a range of biological activities of TRH with potential value in diagnostic and therapeutic measures in humans.

ACKNOWLEDGEMENTS

This work was supported in part by USUHS protocol #R09211 to Dr. Feuerstein. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Departments of Health and Human Services and Defense or the National Institutes of Health and the Uniformed Services University of the Health Sciences. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals", Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. NIH 80-23, 1980). The authors wish to thank Ms. Sharon Benheim for her excellent technical assistance. We also wish to thank Mrs. Laura L. Garza for her help in preparing this manuscript.

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Received 30 April 1987
Accepted 30 April 1987