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[COMMUNICATION]

Effects of Thyrotropin-Releasing Hormone Analogues and Metabolites on Prolactin Release from the Bullfrog Hypophysis

MAKOTO SAKAI, DAISUKE UCHIDA, TADASHI YANAGISAWA[1] and SAKAE KIKUYAMA

Department of Biology, School of Education, Waseda University, Shinjuku-ku, Tokyo 169-50, and [1]Department of Agricultural Chemistry, Utsunomiya University, Utsunomiya, 321, Japan

ABSTRACT—*In vitro* release of prolactin (PRL) from the pituitary gland of the bullfrog (*Rana catesbeiana*) in the presence of thyrotropin-releasig hormone (TRH) analogues and metabolites was studied. [3-Met-His^2]TRH stimulated the release of PRL markedly. The stimulatory activity of the analogue was as potent as that of TRH. In contrast, [1-Met-His^2]TRH had no effect. Two main metabolites of TRH, pyroglutamyl-histidyl-proline and histidyl-proline-diketopiperazine did not affect the release of PRL from the pituitary gland.

INTRODUCTION

In amphibians, thyrotropin-releasing hormone (TRH, pGlu-His-PRoNH$_2$) exhibits potent prolactin (PRL)-releasing activity [1–4], as in mammals [5]. TRH is regarded as the main PRL-releasing factor in the amphibian hypothalamus, since immunoneutralization of TRH in hypothalamic extract with antibody against TRH markedly attenuates its PRL-releasing activity [6], and among the fractions separated chromatographically from the hypothalamic extract, the TRH-containing fraction exhibits the most potent PRL-releasing activity [7].

Recently, presence of [1-Met-His^2]TRH, an analogue of TRH, has been reported in the brain of cyprinid fish [8, 9]. Another analogue [3-Met-His^2]TRH has potent biological activity in mammals [10]. Although these analogues have never been detected in the amphibian tissue [Uchida *et al.*, unpublished], it is of interest to test these substances for PRL-releasing activity in amphibians. In addition, PRL-releasing activity of two main metabolites of TRH, pyroglutamyl-histidyl-proline (TRH-OH) and histidyl-proline-diketopiperazine (Cyclo(His-Pro)) [11] has also been studied. The latter metabolite is known to have various activites related, or opposed to those of TRH in mammals [11, 12].

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MATERIALS AND METHODS

Peptides

TRH, [3-Met-His^2]TRH and TRH-OH were purchased from Bachem Inc. Cyclo(His-Pro) and [1-Met-His^2]TRH were products of Sigma and Peninsula Laboratories, respectively.

Incubation of pituitary gland

Adult bullfrogs (*Rana catesbeiana*) weighing 250–350 g were sacrificed by decapitaion. The anterior pituitary gland was removed, weighed and hemisected and the two hemipituitaries were placed in a glass vial containing 200 μl of 67% Eagle MEM (Nissui Seiyaku Co., Ltd., pH 7.4). The vials were incubated in a Dubnoff metabolic incubator under an atmosphere of 95% O_2-5%

CO_2. After 1 hr of preincubation, the medium was replaced with fresh medium containing a test substance. Incubation was carried out for 16 hr at 25°C, since it had been verified previously that bullfrog pituitaries incubated under the above conditions continue to release PRL until at least 28 hr at a linear rate [4].

Radioimmunoassay

Homologous radioimmunoassay for bullfrog PRL in the medium was performed according to the method described previously [13].

Statistics

Statistical analysis was perfomed by analysis of variance.

RESULTS AND DISCUSSION

TRH stimulated the release of PRL from the pituitary gland dose-dependently (Fig. 1). Likewise, [3-Met-His^2]TRH also showed dose-dependent stimulation of PRL release (Fig. 2). In contrast, [1-Met-His^2]TRH had no significnat effect on the release of PRL from the pituitary gland (Fig. 3).

In cyprinid fish, [1-Met-His^2]TRH seems to stimulate the release of gonadotropin rather than the release of PRL from the pituitary *in vitro* [14]. In the frog hypothalamus, neither [3-Met-His^2]TRH nor [1-Met-His^2]TRH has been detected: High performance liquid chromatography analysis of acid extract of bullfrog hypothalami revealed that none of the fractinos with the same

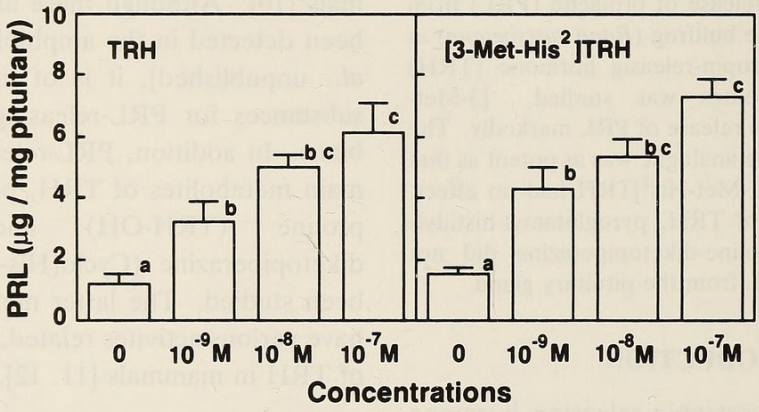

FIG. 1. Effect of TRH (left) and [3-Met-His^2]TRH (right) on release of PRL from the bullfrog pituitary gland *in vitro*. Each column and vertical line represent the mean of 7 determinations and standard error of the mean, respectively. Values with the same superscript do not differ significantly at the 5% level.

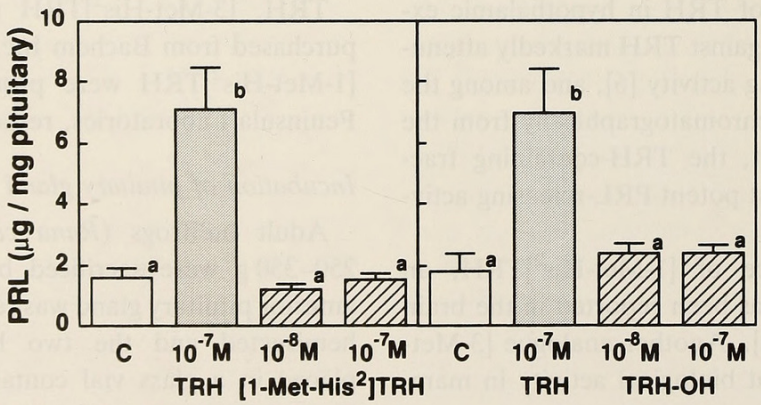

FIG. 2. Effect of [1-Met-His^2]TRH (left) and TRH-OH (right) on release of PRL from the bullfrog pituitary gland *in vitro*. C, control medium. Each column and vertical line represent the mean of 7 determinations and standard error of the mean, respectively. Values with the same superscript do not differ significantly at the 5% level.

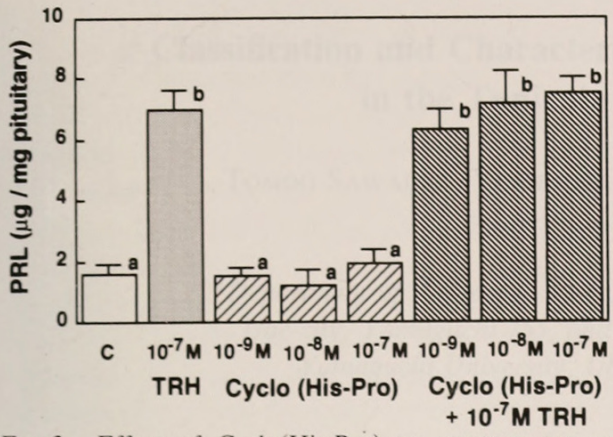

FIG. 3. Effect of Cyclo(His-Pro) on spontaneous or TRH-induced release of PRL form the bullfrog pituitary gland *in vitro*. C, control medium, Each column and vertical line represent the mean of 7 determinations and standard errorr of the mean, respecitvely. Values with same superscript do not differ significantly at the 5% level.

retention time as that of synthetic [3-Met-His^2]TRH or [1-Met-His^2]TRH contain any immunoassayable amount of these substances [Uchida *et al.*, unpublished].

TRH-OH had no significant effect on the release of PRL (Fig. 4). Cyclo(His-Pro) also affected neither the spontaneous nor the TRH-induced release of PRL from the pituitary (Fig. 5). TRH-OH and Cyclo(His-Pro) are the main metabolites of TRH in the brain of vertebrates including amphibians [11]. Data concerning the possible function for TRH-OH are limited [11, 12]. On the other hand, Cyclo(His-Pro) has various TRH-like activities such as antagonism of ethanol narcosis [15], elevation of brain cyclic GMP levels [16] and inhibition of food intake [17] as well as TRH-opposed activities such as hypothermia [18] and inhibition of *in vivo* and *in vitro* PRL secretion [19, 20] in mammals. However, the present experiment with bullfrog pituitary revealed that cyclo(His-Pro) does not affect the release of PRL directly. Among TRH and its related peptides, TRH seems to be the only hypothalamic factor which directly influences the release of PRL in the bullfrog.

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