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Effects of TRH and TRH-Like Peptide pGLU-HIS-GLY-NH_2 on Adrenocortical Cell Proliferation in Rats

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The effects of three peptides—colon mitosis inhibitor (CMI), its amide, and thyroliberin (TRH)—on the adrenocortical cell proliferation were studied. As an index of cell proliferation, the incorporation of bromodeoxyuridine (BrDU) into adrenocortical cell nuclei was used. It was found that: Twelve hours after the injection, only CMI-amide significantly increased the total number of BrDU-immunopositive cell nuclei per equatorial section of the adrenal cortex. This mitogenic effect was observed separately for the entire section, zona glomerulosa, and zona fasciculata. TRH stimulated the cell proliferation of the adrenal cortex 24 h after the injection. Its mitogenic effect was observed for entire section and zona glomerulosa. These findings suggest that these two related peptides, TRH and CMI-amide, should be considered as growth factors for the adrenal cortex.

Key Words: TRH; CMI; proliferation; adrenocortical cells.

Introduction

The growth of the adrenal cortex is controlled by many factors, such as ACTH, some fragments of POMC, angiotensin II, renin, FGF, EGF, and others (Cater and Stack Dunne, 1955; Payet and Isler, 1976; Gospodarowicz et al., 1977; Gill and Simonian, 1977; Lowry et al., 1983). Some of them act as humoral factors, and the others as a paracrine substances. On the other hand, it is known that TRH and TRH-like peptides are widely distributed in animal tissues, including rat adrenal gland (Tal et al., 1984; Mitsuma et al., 1987). So far, the authors investigated the role of TRH only in the regulation of adrenal cortex secretion. It has been found that TRH markedly inhibits the late steps of glucocorticoid synthesis, without affecting earlier steps of this process (Neri et al., 1993). According to our knowledge, there are no reports about the role of TRH in the growth processes in the adrenal cortex. Thus, the aim of our study has been to examine the effect of TRH and TRH-like tripeptide, such as CMI and its amide on the proliferation of adrenocortical cells. CMI is structurally very similar to TRH, from which it differs only by one amino acid residue, namely it contains glycine instead of prolinamide. CMI was isolated from the murine intestinal extracts, and has been found to exert antiproliferative action on the mouse colonic epithelium (Skraastad et al., 1988). Ten years earlier, this tripeptide was extracted from the urine of patients suffering from anorexia nervosa. It possesses anorexogenic properties (Trygstad et al., 1978). TRH is known to stimulate the anterior pituitary cell proliferation (Pawlikowski et al., 1978; Pawlikowski and Słowińska-Klencka, 1994), but it inhibits the proliferation of the pituitary tumoral cell lines GH3 (Yaijima and Saito, 1983), mouse epidermis, and mouse and rat colonic epithelium (Skraastad et al., 1988; Pawlikowski et al., 1993).

Results

Twelve hours after the injection, only CMI-amide significantly increased the total number of BrDU-immunopositive nuclei per equatorial adrenal section, as compared to controls. This proliferogenic effect was observed separately for the entire adrenal section (Fig. 1A), zona glomerulosa (Fig. 1B), and zona fasciculata (Fig. 1C). Twenty-four hours after the injection, the mitogenic effect of CMI-amide seems to still exist, but is not significant. At this point in time, only TRH exerted significantly proliferogenic effect on the adrenal cortex. TRH increased the number of BrDU-immunopositive nuclei both in the entire adrenal equatorial section (Fig. 2A) and in zona glomerulosa (Fig. 2B).

Discussion

The data presented above indicate that TRH and TRH-like peptide CMI-amide increase the cell proliferation of

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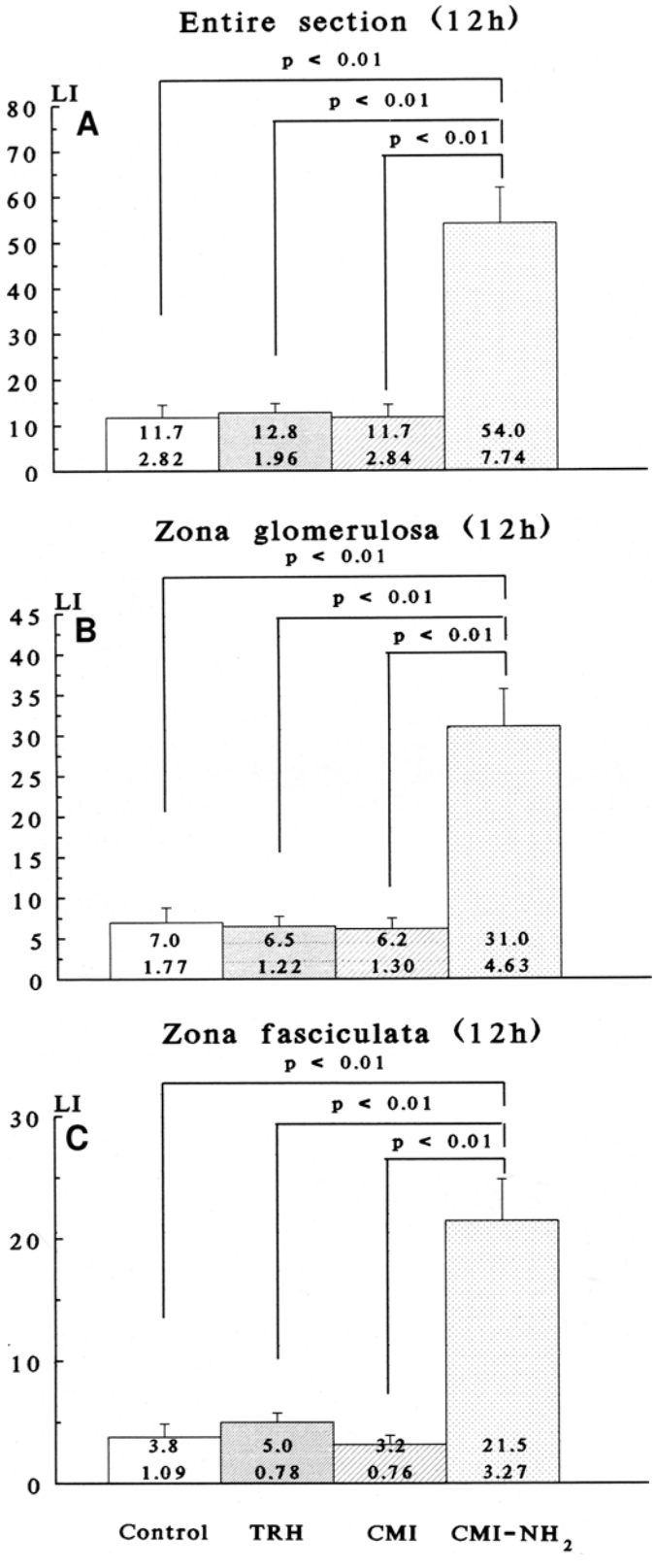

Fig. 1. Effects of TRH, CMI, and CMI-amide on bromodeoxyuridine (BrDU) incorporation into nuclei of adrenocortical cells assessed per equatorial adrenal section, 12 h after the injection of examined substances, **(A)** LI-number of BrDU-immunopositive nuclei per entire equatorial adrenal section. Bars represent means and SEM, p-level of significance. **(B)** LI-number of BrDU- immunopositive nuclei in zona glomerulosa per equatorial adrenal section. **(C)** LI-number of BrDU-immunopositive nuclei in zona fasciculata per equatorial adrenal section.

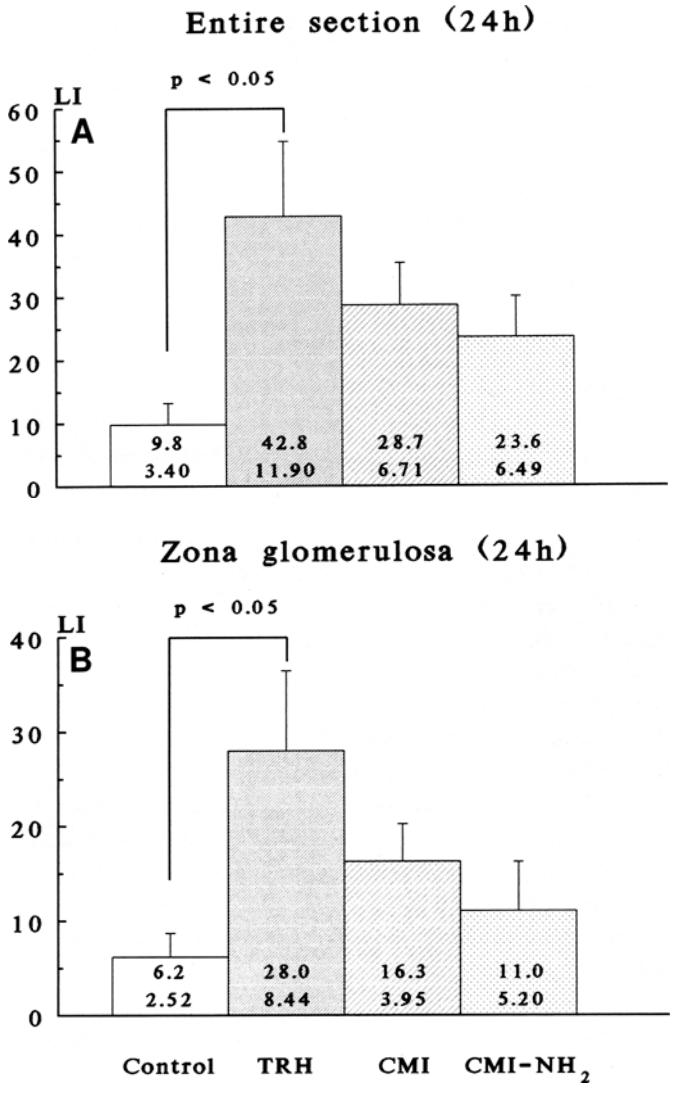

Fig. 2. Effects of TRH, CMI, and CMI-amide on bromodeoxyuridine (BrDU) incorporation into nuclei of adrenocortical cells assessed per entire equatorial adrenal section, 24 h after the injection of examined substances, **(A)** LI-number of BrDU-immunopositive nuclei per entire equatorial adrenal section. Bars represent means and SEM, p-level of significance. **(B)** LI-number of BrDU- immunopositive nuclei in zona glomerulosa per equatorial adrenal section.

the adrenal cortex. These findings suggest that these peptides play a role in the control of growth of the adrenal cortex. The obtained effect of CMI-amide seems to be tissue-specific since this peptide influence on cell proliferation neither in the anterior pituitary lobe (Pawlikowski and Słowińska-Klencka, 1994), nor in the intermediate lobe of the pituitary (Pawełczyk et al., 1996). In contrast to CMI-amide, TRH was found to be stimulatory for pituitary cell proliferation (Pawlikowski and Słowińska-Klencka, 1994; Pawełczyk et al., 1996), but it suppresses the proliferation of intestinal epithelium (Pawlikowski et al., 1993). In spite of the structural similarity of TRH and CMI-amide, the time-course of their effects on the proliferation of

adrenocortical cells is different, which suggests different mechanisms of action. It is possible that TRH could act indirectly, since it is well known that it releases both prolactin (PRL) and thyrotropin (TSH).

Lewiński et al. (1988) have reported that PRL exerts the mitogenic effect upon the adrenal cortex in male Snell mice with hereditary dwarfism. Bartke et al. (1977) have found the increase of adrenal weight in mice and rats to be the effect of experimental hiperprolactinemia. Others have observed that long-lasting treatment with PRL caused the hypertrophy of zona glomerulosa (Mazzocchi et al., 1986). The presence of prolactin receptor on adrenocortical cells has also been reported (Calvo et al., 1981). Another examined substance that could be considered as a growth factor for adrenal cortex is TSH. Lewiński et al. (1988) does not indicate the proliferogenic effect of TSH on the adrenal cortex in rats. However, Zieleniewski (1968) examined the regeneration processes after the enucleation of adrenal gland indicating that TSH stimulated it. Since TRH is present in the adrenal gland (Tat et al., 1984), and the adrenal gland possesses specific TRH binding sites (Bhargava and Gulati, 1988), we cannot exclude that it also influenced the adrenal growth directly, via paracrine action. It is known that TRH inhibits the late steps of steroidogenesis (Neri et al., 1993). It may be that the decrease of corticosterone with supposed subsequent increase of ACTH take part in the proliferogenic effect of TRH on the adrenal gland.

It this study, only CMI-amide exerted the growth-promoting effect upon the adrenal cortex 12 h after the injection. Twenty-four hours after the injection, the mitogenic effect of CMI-amide still seems to persist, but is not statistically significant. At this point in time, the proliferative indices in groups treated with CMI and CMI-amide were very similar. Perhaps, CMI-amide is a directly acting substance on the adrenocortical proliferation, whereas CMI has to transform into amide form to influence the adrenal growth. That is a possible explanation as to why the proliferogenic effect of CMI-amide occurs earlier than that of CMI. The amide group seems to be necessary for the growth effect of this peptide. It is worth recalling that in women suffering from anorexia nervosa, the great amount of CMI in the urine has been observed (Trygstad et al., 1978). Moreover, the alteration in glucocorticoid metabolism in this disease has been reported. Perhaps there is a relationship between elevated urine level of CMI, and growth and function of the adrenal gland in this disease. On the other hand, CMI, in contrast to TRH, is ineffective in stimulation of PRL and TSH release (Pawlikowski et al., 1993). CMI-amide was also ineffective in stimulating TSH secretion (unpublished results from our laboratory). Thus, a direct action of these peptides on the adrenal cortex could be rather presumed. However, this hypothesis needs further confirmation in the in vitro studies. Moreover, the nature of the receptors involved remains to be clarified.

Materials and Methods

Adult male Wistar rats weighing 200 ± 10 g were used in the study. The animals were kept under controlled light conditions (12 h light, 12 h darkness) with food and water *ad libitum*. All the animals were divided into four groups and received single injections of the following substances subcutaneously: Group I-Control, 0.9 % NaCl; Group II-TRH (Berlin-Chemie AG); Group III-Colon mitosis inhibitor (CMI, pGlu-His-Gly acetate, Sigma, St. Louis, MO); Group IV-CMI-amide (Sigma). All these peptides were injected at a dose of 100 μg/kg b.w., in vol 0.2 mL; at 20.00. The rats of all groups (eight animals in each group) were killed by decapitation 12 and 24 h after the injection. Two hours before decapitation, each animal received an intraperitoneal injection of bromodeoxyuridine (BrDU, Sigma) at a dose of 50 mg/kg b.w. The adrenal glands were collected from each animal and fixed in Bouin-Holland fluid. Tissues were embedded in paraffin wax, and the paraffin sections were immunostained using cell proliferation kit (Amersham International, Amersham, Bucks, UK) to detect BrDU incorporated into adrenocortical nuclei. The cell proliferation was assessed according to the method of Michat and Nouët, (1975). All serial sections were estimated, the largest (equatorial) being chosen for counting. The number of BrDU-immunopositive nuclei was estimated. We counted the total number of BrDU-immunopositive nuclei per equatorial section of the adrenal, and per each zona separately (zona glomerulosa, fasciculata, reticularis). The data were analyzed statistically, and the significance of differences between medians of number of BrDU-immunopositive nuclei were determined by Mann-Whitney's test.

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