Vol. 202, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1994 Pages 155-160

IN VITRO EFFECTS OF CINC/GRO, A MEMBER OF THE INTERLEUKIN-8 FAMILY, ON HORMONE SECRETION BY RAT ANTERIOR PITUITARY CELLS

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Received May 21, 1994

We investigated the effects of CINC/gro on hormone secretion using normal rat anterior pituitary cells. In normal anterior pituitary cells, 10-100 ng/ml of CINC/gro significantly increased the secretion of PRL within 3 h of incubation, and two-fold enhancement of PRL secretion was induced by 100 ng/ml of CINC/gro within 24-h incubation, while the response of GH and ACTH secretions to CINC/gro was weak. On the other hand, CINC/gro suppressed basal LH and FSH secretions in a concentration-dependent manner. The percent inhibition of basal secretion by CINC/gro (50 ng/ml) within 24-h incubation was 70% for LH and 43% for FSH. Twenty-four-hour incubation with 100 ng/ml of IAP completely blocked the CINC/gro-stimulated PRL and GH secretions and CINC/gro's suppression of both basal LH and FSH secretions. These data demonstrate a new biological activity for CINC/gro and provide evidence for immune system regulation of anterior pituitary hormone secretion.

There is increasing evidence that the neuroendocrine and immune systems are closely linked. Neuroendocrine hormones such as PRL and GH have recently been shown to stimulate immune functions both *in vivo* and *in vitro* (1,2). On the other hand, cytokines, such as interleukin-1 (IL-1), TNF-α and IL-6, influence pituitary hormone release (3-6).

Among the cytokines, interleukin-8 (IL-8) is a key mediator in the migration of neutrophils from the circulation to sites of inflammation in the tissues (7,8). IL-8 is structurally and functionally related to several members of the macrophage inflammatory protein-2 (MIP-2) family of cytokines. These include MIP-2, gro/MGSA (growth-related oncogene/melanoma growth-stimulating activity) and neutrophil-activating peptide 2 (NAP-2)(9).

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Abbreviations: CINC/gro, cytokine-induced neutrophil chemoattractant/growth-related oncogene; TNF-α ,tumor necrosis factor-α; ELISA, enzyme-linked immunosorbent assay ; IAP, islet-activating protein.

0006-291X/94 $5.00

Stimulation of neutrophils with IL-8, NAP-2 or gro/MGSA causes intracellular calcium mobilization and elicits motile, secretory and metabolic responses (10) that are critical to the roles of neutrophils in host defenses.

Recently, a CINC/gro, an IL-8-like neutrophil chemoattractant, was purified and cloned(11). The primary structure of CINC/gro indicates that CINC/gro is the rat counterpart of human GRO (12), and therefore belongs to the IL-8 family. Although there have been many studies focusing on the biochemistry of the IL-8 family (9,13), the roles of the IL-8 family in the pituitary gland have not yet been clarified with regard to the cross-talk mechanism between the immune and neuroendocrine systems.

In the present study, we have examined the effects of CINC/gro on hormone secretion by rat normal anterior pituitary cells. We also investigated the effects of IAP, inducer of ADP ribosylation of Giα, which is the G protein linked to inhibition of adenylase cyclase, on CINC/gro-induced hormonal responses.

Materials & Methods

Cell culture: Normal anterior ptuitary cells were obtained from female Wistar rats (200-250 g) and dispersed enzymatically as described previously (14). The dispersed cells were seeded at a density of 0.7 X 10^6 viable cells/well and allowed to attach for at least 4 days in a humidified 37°C atmosphere of 5% CO_2 and 95% air. For the experiments on hormone secretion, the cells were washed twice and cultured in serum-free RPMI-1640 medium (Handai Biken, Osaka, Japan) or specially designed media containing various concentrations of CINC/gro. After designated incubation times, the conditioned media was collected from the wells and stored at -20°C until hormone assay. In the experiments on IAP, cells were preincubated for 3 h with the serum-free medium with or without 100 ng/ml of IAP. Then the medium was replaced with the serum-free medium with or without 100 ng/ml of IAP containing various concentrations of CINC/gro (5-100 ng/ml). Throughout the experiments, the cell morphology was observed by phase-contrast optics. 100 ng/ml of IAP had no effects on the cell viability, as determined by the trypan blue exclusion test, the cell morphology or the attachment of the cells to the plastic culture dish.

RIA: PRL, LH, FSH and GH were determined by double-antibody radioimmunoassays using the materials and protocols supplied by NIADDK of the National Institutes of Health. The results are expressed in terms of standard PRL-RP-3, LH-RP-3, FSH-RP-2 and GH-RP-2. TSH and ACTH were determined with a rat TSH radioimmunoassay kit using the materials and protocols supplied by Amersham International plc (Amersham, England) and a rat ACTH radioimmunoassay kit by Mitsubishi-yuka (Tokyo, Japan). All samples were assayed in duplicate. The intra- and interassay variations for each of the six hormones were less than 8% and 10%, respectively.

Materials: Rat CINC/gro (Peptide Institute, Inc., Osaka, Japan) and IAP (List Biological; Campbell, CA, USA) were dissolved directly in RPMI-1640 medium to the desired concentrations. All other chemicals were commercial materials of the highest purity available and were used without further purification.

Statistical analysis: In this study, each data point represents the mean ± SEM of independent experiments. All data were subjected to analysis of variance, and differences between groups were assessed using the multiple range test of Duncan. A p value of less than 0.05 was considered to represent a statistically significant difference.

Results

CINC/gro stimulated PRL and GH secretion from anterior pituitary monolayer cultures in a concentration-dependent manner (Figures 1 & 2). CINC/gro also stimulated ACTH (at 100 ng/ml, $P<0.05$)but not TSH secretion within 24 h of incubation. As shown in Figure 1, 10-100 ng/ml of CINC/gro significantly increased the secretion of PRL within 3 h of incubation, and this effect continued throughout the first 24 h of incubation. Two-fold enhancement of PRL secretion was induced by 100 ng/ml of CINC/gro within 24 h of incubation, while the response of GH and ACTH secretions to CINC/gro was weak. On the other hand, CINC/gro suppressed the basal LH and FSH secretions from normal anterior pituitary cells in a concentration-dependent manner (Figures 3 & 4). This inhibitory effect of CINC/gro on the LH and FSH secretions was observed within 3 h and continued throughout the first 24 h of incubation. This inhibition of the LH and FSH secretions by CINC/gro reached to a minimum at a CINC/gro concentration of 10 ng/ml. The percent inhibition of the LH and FSH secretions within 24 h of incubation by CINC/gro (50 ng/ml) was 70% for LH and 43% for FSH. The IC_{50} of CINC/gro for the LH and FSH secretions within 24 h of incubation was 3.6 ng/ml for LH and 4.6 ng/ml for FSH. No effects of CINC/gro on pituitary hormone secretions were observed until after 3 h of incubation.

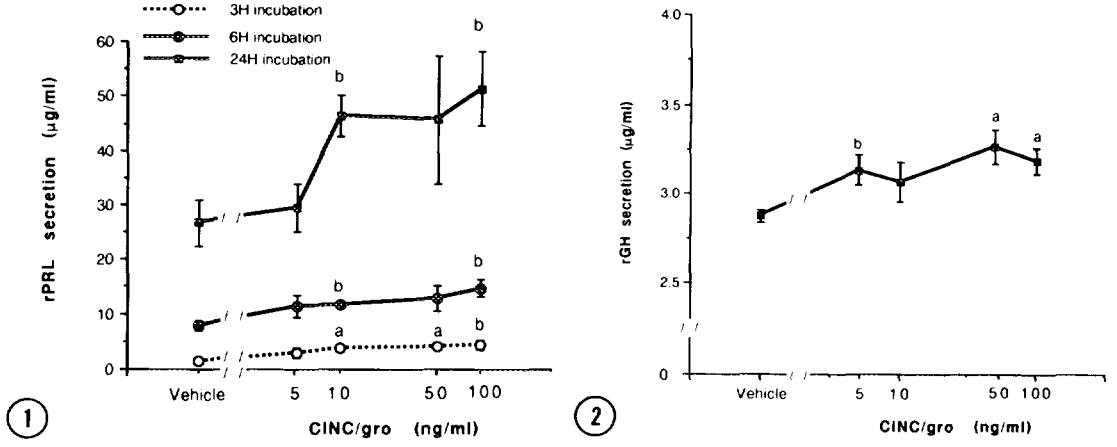

Figure 1. Time and dose responses of CINC/gro-induced PRL secretion by rat normal anterior pituitary cells. The cells were incubated with serum-free growth medium containing various concentrations of CINC/gro (5-100 ng/ml) for the designated incubation times (3 h, 6 h and 24 h). The values are the mean ± SEM of four independent experiments.
a $P<0.01$ vs. vehicle at each incubation time.
b $P<0.05$ vs. vehicle at each incubation time.

Figure 2. Dose response of CINC/gro-induced GH secretion by rat normal anterior pituitary cells. The cells were incubated with serum-free growth medium containing various concentrations of CINC/gro (5-100 ng/ml) for 24 h. The values are the mean ± SEM of four independent experiments. See Materials and Methods for other details.
a $P<0.01$ vs. vehicle b $P<0.05$ vs. vehicle

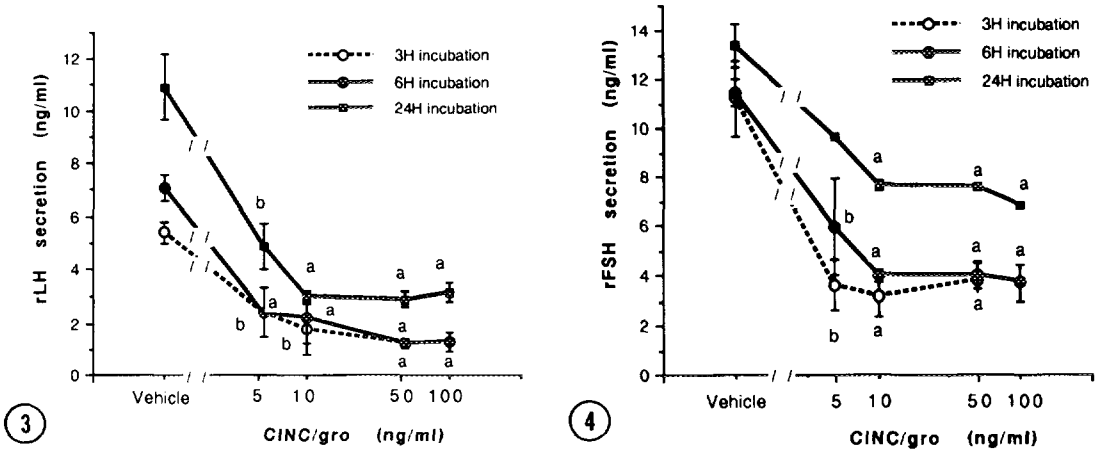

Figure 3. Time and dose effects of CINC/gro on LH secretion by rat normal anterior pituitary cells. The cells were incubated with serum-free growth medium containing various concentrations of CINC/gro (5-100 ng/ml) for the designated incubation times (3 h, 6 h and 24 h). The values are the mean ± SEM of four independent experiments. See Materials and Methods for other details.
a $P<0.01$ vs. vehicle at each incubation time.
b $P<0.05$ vs. vehicle at each incubation time.

Figure 4. Time- and dose-dependent effects of CINC/gro on FSH secretion by normal anterior pituitary cells. The cells were incubated with serum-free growth medium containing various concentrations of CINC/gro (5-100 ng/ml). The values are the mean ± SEM of four independent experiments. See Materials and Methods for other details.
a $P<0.01$ vs. vehicle at each incubation time.
b $P<0.05$ vs. vehicle at each incubation time.

Twenty-four-h incubation with 100 ng/ml of IAP completely blocked the CINC/gro (50 & 100 ng/ml) stimulated PRL and GH secretions (Table 1). Twenty-four-h incubation with 100 ng/ml of IAP also completely blocked the CINC/gro (10 and 100 ng/ml) mediated suppression of both the LH and FSH secretions. As shown in Table 1, 24-h incubation with 100 ng/ml of IAP had no effects on basal PRL, GH, LH or FSH secretion.

Discussion

Information is rapidly accumulating which supports the existence of a close linkage between the immune and endocrine systems. There are numerous reports of the influences of cytokines on pituitary hormone release (3-6,15). In the present study, CINC/gro stimulated the secretion of PRL, GH and ACTH but suppressed the secretion of LH and FSH from rat anterior pituitary cells. To the best of our knowledge, this is the first report to demonstrate that (1) CINC/gro influenced anterior pituitary hormone release, and (2) its action is mediated through the IAP-sensitive G protein.

It is noteworthy that CINC/gro induced the secretion of PRL, GH and ACTH, but suppressed secretion of LH and FSH, while many other cytokines such as IL-1, IL-6 and TNF-α caused multiple hormone release from anterior pituitary cells

Table 1 Effect of IAP on hormone secretion induced by CINC/gro

	CINC/gro concentration (ng/ml)	Secreted Concentration	
		IAP (-)	IAP (+)
PRL (μg/ml)	Vehicle	30.15 ± 1.22	30.70 ± 3.41
	50	47.21 ± 0.69 [a]	32.66 ± 2.54 [b]
	100	52.10 ± 3.11 [a]	31.39 ± 3.06 [b]
GH (μg/ml)	Vehicle	2.88 ± 0.04	2.96 ± 0.12
	50	3.27 ± 0.09 [a]	2.92 ± 0.11 [b]
	100	3.19 ± 0.07 [a]	3.08 ± 0.15 [b]
LH (ng/ml)	Vehicle	9.66 ± 0.51	10.89 ± 0.43
	10	4.82 ± 0.85 [a]	11.85 ± 1.55 [b]
	100	5.36 ± 0.80 [a]	11.83 ± 0.65 [b]
FSH (ng/ml)	Vehicle	13.40 ± 0.04	13.61 ± 0.08
	10	7.81 ± 0.06 [a]	13.65 ± 0.12 [b]
	100	8.01 ± 0.04 [a]	13.66 ± 0.11 [b]

Effects of IAP on hormone secretion induced in normal anterior pituitary cells by CINC/gro. The values are the mean ± SEM of four independent experiments.
a $P<0.01$ vs. vehicle with or without IAP.
b $P<0.01$ vs. IAP-free sample containing the same concentration of CINC/gro.

within 30 min(3,4,15). In contrast with the rapid action of these cytokines , the effect of CINC/gro on PRL, GH and ACTH secretions has a substantial lag time (approximately 24 h), while the effect of CINC/gro on LH and FSH secretions is rapid (3 h). At present, we cannot give any explanations for these discrepant responses. Further studies are necessary to elucidate the mechanisms of these discrepant responses to CINC/gro's action.

IL-8 is structurally and functionally related to gro/MGSA, which appears to share a receptor on neutrophils with IL-8 (9,16,17). Recent studies revealed that the IL-8 receptor from human neutrophils is a member of the superfamily of G protein-linked receptors that contain seven transmembrane domains, and that stimulation of neutrophils with IL-8 or gro/MGSA caused intracellular calcium mobilization (10,18,19) and secretory responses such as release of storage proteins (20). It has also been reported that all responses to NAP-1 and other chemotaxins were inhibited by pretreatment with IAP (10). Taken together, the blockade by IAP of the effect of CINC/gro on anterior pituitary hormone secretion suggests that the action of CINC/gro appears to be, in part, mediated by an IAP-sensitive G protein.

CINC/gro may be produced in the anterior pituitary, because we detected CINC/gro immunoreactivity in the anterior pituitary gland by immunohistochemistry and immunoblotting and we also detected significant immunoreactive CINC/gro in the conditioned medium from dispersed anterior pituitary cells by ELISA(submitted for publication). Histochemical studies are under way to clarify the nature of the CINC/gro-producing cells.

In conclusion, newly-described biological activity reported here adds to the growing list of activities attributed to CINC/gro and suggests the possibility that CINC/gro may have some actions as a pituitary hormone modulator in the cross-talk mechanism between the immune and neuroendocrine systems.

Acknowledgments

We thank the NIADDK, Bethesda, MD, USA, for their generous gifts of rat PRL, GH, LH and FSH RIA kits. This work was supported by a grant for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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