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SYNERGISTIC EFFECTS OF CORTICOTROPIN AND INSULIN-LIKE GROWTH FACTOR I ON CORTICOTROPIN RECEPTORS AND CORTICOTROPIN RESPONSIVENESS IN CULTURED BOVINE ADRENOCORTICAL CELLS

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SUMMARY. Pretreatment of bovine adrenocortical cells with increasing concentrations of insulin-like growth factor I (IGF-I) for 3 days resulted in a dose dependent ($ED_{50} \simeq 5$ ng/ml) increment in Corticotropin (ACTH) receptors. Moreover, IGF-I pretreatment potentiated the effects of maximal active concentration of ACTH (10^{-9} M) on its own receptors. Whereas ACTH (10^{-9} M) or IGF-I (50 ng/ml) alone induced a 3- and 2.5-fold increase respectively in ACTH receptors, there was a 7.5 fold increase in the presence of the two peptides. This synergism between ACTH and IGF-I was also observed for the ACTH-induced cortisol response with an increase of 9-, 3- and 20-fold for cells pretreated with ACTH, IGF-I and the two peptides, respectively. However, the effects of both peptides on ACTH-induced cAMP production was only additive. The present results show that ACTH and IGF-I are potent stimulating factors on bovine adrenal cell differentiated functions and that the effects of both peptides are synergistic.

Corticotropin (ACTH) is the main hormone which in mammals regulates not only the acute glucocorticoid secretion but also the expression and maintenance of adrenal cell specific functions, namely ACTH receptor number (1) and the expression of the genes encoding several enzymes involved in the steroidogenic pathway (2). Moreover, recent studies indicate that insulin-like growth factor I (IGF-I), which alone has no steroidogenic activity, plays a key role in the maintenance of adrenal cell differentiated functions (3, 4). In the present work, we have studied whether IGF-I regulates ACTH receptor number and whether this growth factor has an additive or synergistic effect with ACTH on the regulation of adrenal cell specific functions.

MATERIALS AND METHODS

Pure recombinant DNA IGF-I was supplied by Kabi-Vitrum (Stockholm, Sweden). ACTH (Synacthen) was obtained from Ciba (Rueil-Malmaison, France), Ham's F12 medium and Dulbecco's modified Eagle's medium (F12/ DMEM), nystatin, penicillin/streptomycin, trypsin/EDTA and fetal calf serum from Gibco (Paris, France), trypsin from Biomérieux (Lyon, France). ^{125}I labeled human (Tyr^{23})-ACTH-(1-39) (^{125}I-ACTH), specific activity ≃ 74 TBq/mmol, was obtained from Amersham International (U.K.) and the other reagents from Sigma.

Bovine adrenocortical fasciculata cells were prepared by sequential incubation with trypsin (0.15%) in an equal volume of F12/DMEM containing antibiotics and

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cultured in a serum-free medium (5, 6). On the second day of culture, the medium was removed and replaced with fresh medium alone or with the factors indicated for 72 h. (^{125}I)- ACTH binding studies were performed as described elsewhere (1). cAMP and cortisol contents of the media were determined by specific RIAs (3, 6). At the end of each experiment, the number of cells was measured (Coulter, ZBI). Statistical analyses were performed with Student's t-test for comparison of two groups. Differences were considered significant when $p < 0.05$. All experiments were realized at least three times with different cell preparations.

RESULTS AND DISCUSSION

Pretreatment of bovine adrenocortical cells for 3 days with increasing concentrations of IGF-I induced an increase of ^{125}I -ACTH binding in a dose-dependent manner ($ED_{50} \simeq 5$ ng/ml, Vmax ≃50 ng/ml) (Fig. 1). This effect was due to a rise in the number of high affinity binding sites per cell (from 2570 ± 490 to 5320 ± 720 sites per cell n = 3) without modification of their affinity (Fig. 1, insert). These results indicate that IGF-I, which enhances 2- to 3-fold the A-II receptor number (3), has also a positive effect on ACTH-receptor number. The data in Fig. 2 confirm that pretreatment of adrenal cells with IGF-I enhanced in a dose-dependent manner the cAMP and cortisol response to ACTH (3). Since ACTH regulates positively its own receptors and the cAMP and cortisol response to further hormonal stimulation (1), we examined the effects of IGF-I and maximal active concentration of ACTH (10^{-9}M) on ACTH receptors and ACTH responsiveness (Fig. 2). The effects of ACTH on its own

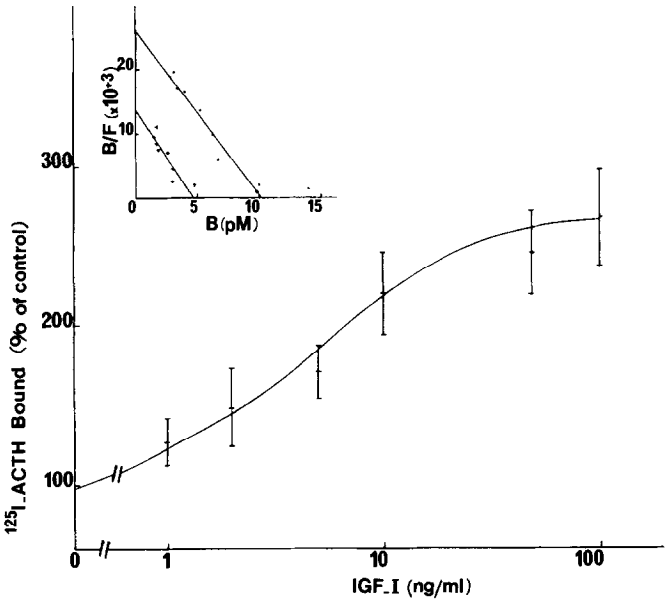

Fig. 1. Dose-response effects of IGF-I on ACTH receptors in bovine adrenocortical cells. Cells were treated with the indicated concentrations of IGF-I for 72 h. The hormone was added daily. At the end of the incubation period, the specific binding of (^{125}I)-ACTH was measured. The results, expressed as percent of control (cells incubated with medium alone), are mean ± SEM of five experiments, each done in triplicate.
Insert : Scatchard analysis of the binding data for control (+) and IGF-I (50 ng/ml) (O) pretreated cells.

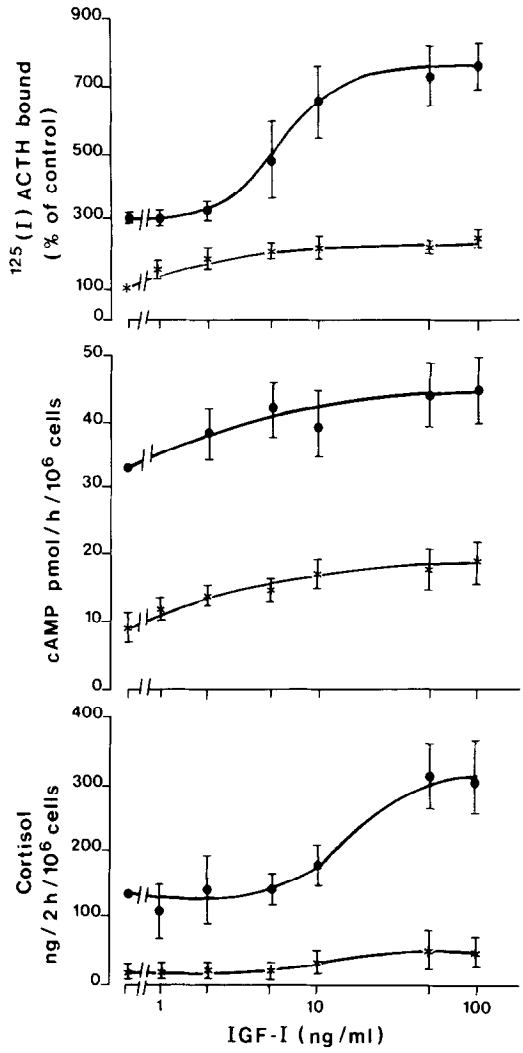

Fig. 2. Synergistic effects of IGF-I and ACTH on ACTH receptors and ACTH responsiveness in bovine adrenocortical cells. Cells were treated with increasing concentrations of IGF-I in the absence (✱) or presence of ACTH (10^{-9}M) (●) for 72 h. The medium was removed, the cells were washed with acidic glycin buffer and the binding of (^{125}I)-ACTH (A), acute cAMP response (B) and acute cortisol response (C) to 10^{-9}M ACTH were determined. The results are the mean ± SEM of three experiments, each done in triplicate.

receptors were potentiated in a dose-dependent manner by IGF-I (Fig. 2A). At maximal concentrations of IGF-I (50 ng/ml), there was about a 7.5-fold increase whereas ACTH or IGF-I alone induced a 3- and 2.5-fold increase respectively. The pretreatment with both peptides also enhanced the cAMP response to further hormonal stimulation (Fig. 2B and Table 1), but for this response the effects were not synergistic but additive. This lack of synergistic effect might be related to some increase of phosphodiesterase activity following incubation with the two peptides. In fact, the cAMP response in the presence of phosphodiesterase inhibitor (1-methyl-3 isobutylxanthine = MIX) of ACTH and IGF-I pretreated cells was more than additive when compared to the response of cells pretreated with ACTH or IGF-I alone

TABLE 1. Effect of 1-methyl-3-isobutylxanthine (MIX) on ACTH-induced cAMP production in bovine adrenocortical cells

	cAMP production pmoles/h/10^6cells	
	- MIX	+ MIX (5×10^{-4}M)
Control	12 ± 1	31 ± 5
IGF-I 50 ng/ml	21 ± 6	47 ± 7
ACTH 10^{-10}M	31 ± 3	70 ± 2
IGF-I 50 ng/ml + ACTH 10^{-10}M	56 ± 9	145 ± 13

Cells were pretreated with the effectors indicated for 72 h. After the incubation period, the cells were washed with acidic glycin buffer and incubated with ACTH 10^{-9}M. cAMP was determined after 1 h. Values are the mean ± SD of triplicate determinations of three different wells.

(Table 1). On the other hand, IGF-I had a marked synergistic effect on ACTH-induced steroidogenic response to further hormonal stimulation (Fig. 2C). Thus, pretreatment with both factors induced a 20-fold increase of the steroidogenic response to ACTH, whereas pretreatment with ACTH or IGF-I alone enhanced only 9 and 3 times the steroidogenic response. The mechanisms underlying the synergistic effect are not well understood but might be related in part to the different trophic effects of the two peptides on adrenal steroidogenesis. ACTH enhanced the expression and the activity of P_{450}-17 α-hydroxylase and to a lesser extent the expression and activity of the other steroidogenic enzymes (2). On the other hand, IGF-I enhanced the cholesterol available for steroidogenesis and the activities of 3 β-hydroxysteroid dehydrogenase isomerase, 21-hydroxylase and 11β -hydroxylase but had no effect on the activity of 17 α-hydroxylase (3).

Taken together, the above results, those previously published (1, 3, 4) and the recent observations showing that ACTH regulates positively IGF-I receptors on bovine adrenal cells (7) indicate the existence of a reciprocal trophic effect between ACTH and IGF-I on the regulation of their own receptors on bovine adrenal fasciculata cells. These interactions lead to an increased expression of the differentiated functions of these cells and therefore to an increased steroidogenic capacity. A reciprocal trophic effect between IGF-I and other polypeptide hormones has also been reported in two other steroidogenic cells : Leydig cells and granulosa cells. In cultured pig Leydig cells, IGF-I enhanced 3-to 4-fold hCG receptor number and hCG induced a 2-to 3-fold increase of IGF-I receptors within 2 days (8). Similarly, in granulosa cells, IGF-I potentiated the effect of FSH on LH receptors (9) and LH steroidogenic responsiveness (10) and FSH enhanced IGF-I receptors (11). Finally, the role of IGF-I in the physiological regulation of the specific function of steroidogenic cells is strengthened by the fact that this peptide is produced locally in the ovary (12, 13), the testis (14, 15) and the adrenal (16) and that this secretion is regulated in part by the specific trophic hormones.

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