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REGULATION OF IGF-I RECEPTORS BY CORTICOTROPIN AND ANGIOTENSIN-II IN CULTURED BOVINE ADRENOCORTICAL CELLS

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SUMMARY. The effects of angiotensin II (A-II) and corticotropin (ACTH) on insulin-like growth factor-I (IGF-I) receptors of bovine adrenocortical cells were investigated. Pretreatment of cells for 48 h with ACTH or A-II induced in a dose-dependent manner an increase in ^{125}I-IGF-I binding (ED50 $\simeq 10^{-11}$ M, Vmax = 10^{-10} M with ACTH ; ED50 $\simeq 3.10^{-9}$ M, Vmax = 10^{-7} M with A-II). This resulted from an increase in the number of binding sites without modification of the binding affinity. Pretreatment with 8-Bromo-cAMP (10^{-3} M), a phorbol ester (PMA 10^{-7} M) + ionophore A23187 (10^{-7} M) produced a positive regulation of IGF-I receptors. Glucocorticoids did not mediate the effect of A-II and ACTH on IGF-I receptors. Since previous studies have shown that IGF-I increased ACTH and A-II receptors the present data indicate the existence of a reciprocal positive trophic effect between IGF-I and the two hormones on the regulation of their specific membrane-bound receptors.

Insulin-like growth factor-I (IGF-I), a 70-amino acid peptide, appears to be involved not only in the regulation of mammalian growth, but also in the differentiation of several cell types (1, 2). To elicit its biological action, IGF-I binds to its own specific cell surface receptors which have been found in a wide variety of cell types (2) including bovine adrenocortical cells (3). In this cell model, IGF-I plays an important role in the maintenance of several specific differentiated functions, which lead to an enhanced steroidogenic response to both ACTH and angiotensin II (A-II) (3), the two main hormones which control adrenal steroidogenesis. The aim of the present study was to investigate the regulation of IGF-I receptors by A-II and ACTH on bovine adrenocortical cells in primary culture.

MATERIALS AND METHODS

Pure recombinant DNA IGF-I was supplied by Kabi-Vitrum (Stockholm, Sweden). ACTH (Synacthen) was obtained from Ciba (Rueil-Malmaison, France) ; synthetic

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(Ile^5)-angiotensin-II (A-II) from Bachem (Bubendorf, Switzerland) ; Ham's F-12 medium and Dulbecco's modified Eagle's medium (DMEM), nystatin, penicillin/streptomycin, trypsin/EDTA and fetal calf serum from Gibco (Paris, France) ; trypsin from BioMérieux (Lyon, France) ; dexamethasone (Soludecadron) from Merck Sharp-Dohme Chibret (Paris, France) and Na-^{125}I (carrier free) from Amersham International (UK) and the other chemicals were from Sigma Chemicals Co (St Louis, MO, USA). IGF-I was iodinated by the fractional chloramine T method, with specific activities of 250 to 450 uCi/ug and the iodinated peptide was purified on Sephadex G-50.

Bovine adrenocortical cells were prepared by sequential incubation with trypsin (0.15 %) in an equal volume of F12-DMEM containing antibiotics. Isolated cells were washed and then cultured in 4 cm^2 multiwell dishes as previously described (4) except that the medium was not supplemented with insulin. Most of the experiments reported were studied on day 3 of culture.

At the end of the experimental period, the culture medium was aspirated and replaced by F-12/DME medium containing [^{125}I]IGF-I (10^{-10}M), 0.5 % bovine serum albumin and 0.1 % bacitracin in a final volume of 0.5 ml. After a 3 h incubation at 37°C, the cells were washed three times with 150 mM ice-cold NaCl and dissolved in 0.5M NaOH, 0.4 % deoxycholate. Then the radioactivity was measured in a gamma counter with 75 % efficiency. Specific binding was determined by subtracting from the total binding the radioactivity associated with cells in the presence of IGF-I (200 ng) or insulin (100 µg) which give a similar non-specific binding.

The net amounts of [^{125}I]IGF-I bound varied from one cell preparation to another, but the percent increase over the control was less variable. For this reason, all the results are expressed as percent of control. Student's t test for comparison of two groups was used. Differences were considered significant when $P < 0.05$ (the analysis was carried out within each experiment considering results of [^{125}I]IGF-I binding in cpm).

RESULTS AND DISCUSSION

Bovine adrenal cells contain specific IGF type I receptors which have been identified by binding and affinity cross-linking experiments (3). Pretreatment of cells for two days with increasing concentrations of ACTH or A-II induced an increase of [^{125}I]IGF-I binding in a dose-dependent manner (Fig. 1). Half-maximal and maximal effects were observed at about 10^{-11}M and 10^{-10}M for ACTH and at 3×10^{-9}M and 10^{-7}M for A-II. Further increases in the concentration of the two hormones reduced the binding of [^{125}I]IGF-I. Scatchard analysis of the binding data after pretreatment of cells with ACTH (10^{-10}M) or A-II (10^{-7}M) revealed that the observed increase in [^{125}I]IGF-I binding resulted from an enhancement in the number of binding sites (7,500 $\pm$ 400 ; 9,900 $\pm$ 1,300 and 11,700 $\pm$ 2,500 for control, ACTH- and A-II-treated cells, n = 3) without modification of the binding affinity (K_D = 2.5 $\pm$ 0.3 ; 2.2 $\pm$ 0.3 and 2.2 $\pm$ 0.2 10^{-9}M for control, ACTH- and A-II-treated cells, n = 3). The effects of both hormones were time-dependent, the maximal effects were observed between 48 and 72 h.

These results point to the existence of a reciprocal positive trophic effect between IGF-I and the specific peptidic hormones of adrenal cells on the regulation of their specific receptors. Thus, IGF-I produces a 2- to 3-fold increase in both A-II (3) and ACTH receptors (unpublished data). On the other hand, both hormones enhance IGF-I receptors. Moreover, both ACTH and A-II stimulate IGF-I secretion by adrenal cells (5). Therefore, it is likely that the observed effects of both hormones on IGF-I

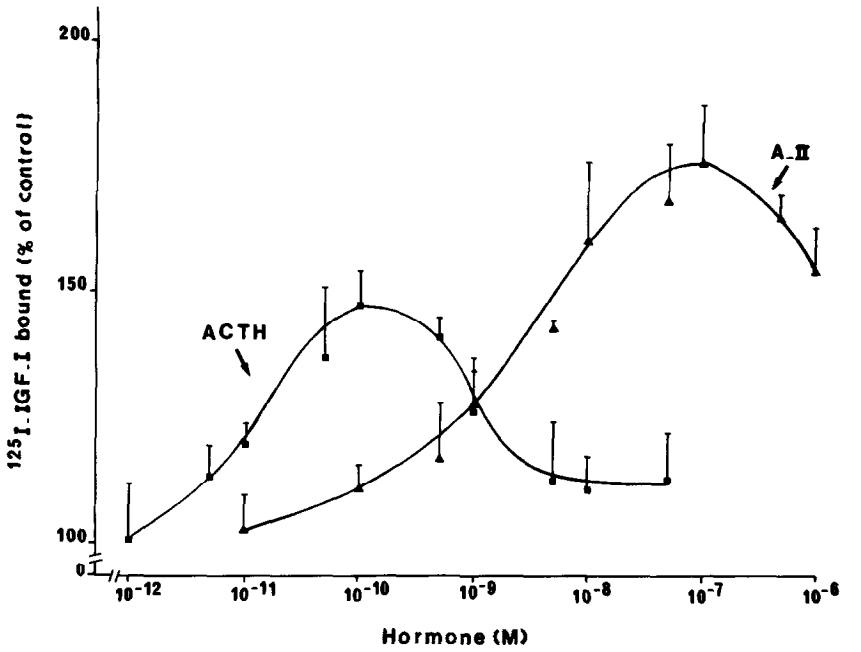

Figure 1. Dose-response effects of A-II and ACTH on (^{125}I)IGF-I binding to bovine adrenal cells. Bovine adrenal cells were incubated for 48 h at 37°C with increasing concentrations of A-II or ACTH. At the end of incubation, the medium was removed and the specific binding of (^{125}I) IGF-I was measured. The results, expressed as percent of control (cells incubated in medium alone), represent the mean ± SD of three experiments, each done in triplicate.

receptors were underestimated, either because the receptor might be occupied or down-regulated by the peptide secreted by the cells. The first hypothesis was ruled out because washing of treated cells with glycine acidic buffer (6), which removes more than 98 % of the bound peptide, did not increase the number of receptors. On the other hand, the second hypothesis is more likely, since IGF-I induces down-regulation of its own receptors in adrenal cells with half-maximal and maximal effect at 10 and 100 ng/ml, concentrations which are in the range of those secreted by adrenal cells following stimulation with ACTH and A-II (5). Thus, the higher stimulatory effects of A-II than ACTH on IGF-I receptors ($P < 0.05$) could be related to the fact that ACTH is a more potent stimulator of IGF-I secretion by cultured cells than A-II, even though the maximal steroidogenic effects are similar (4). Similarly, the additive effects of ACTH and A-II on IGF-I secretion (5) might explain why the number of IGF-I receptors was lower after treatment of cells with both hormones than after treatment with each hormone alone (Table 1). Activation of the cAMP pathway by 8 Bromo-cAMP or the phosphatidylinositide pathway by phorbol ester plus calcium ionophore partially reproduced the effects of ACTH and A-II (Table 1). Again, treatment of cells with both 8 Bromo-cAMP and A-II reduced the (^{125}I)IGF-I binding when compared to the effects of each effector alone (Table 1).

Table 1. Effects of several effectors on (^{125}I) IGF-I binding to bovine adrenal cells

Pretreatment	(^{125}I)IGF-I bound (% of control)	
	Control	A-II 10^{-7}M
Control	100	166 ± 7 (n = 6)
ACTH 10^{-10}M	138 ± 2 (n = 5)	115 ± 7 (n = 5)
8 Bromo-cAMP 10^{-3}M	114 ± 3 (n = 9)	76 ± 5 (n = 4)
8 Bromo-cAMP $5x10^{-3}$M	74 ± 7 (n = 5)	
PMA 10^{-7}M + Ionophore 10^{-7}M	131 ± 4 (n = 4)	

Bovine adrenal cells were incubated for 48 h at 37°C with ACTH (10^{-10}M), 8Br-cAMP (10^{-3}M or $5x10^{-3}$M), PMA (10^{-7}M + ionophore (10^{-7}M) in the absence or presence of A-II (10^{-7}M). Then the medium was removed and the specific binding of (^{125}I)IGF-I was determined. Results, expressed as percent of control, represent the mean ± SEM of the number of experiments (n) indicated, each done in triplicate.

Since both ACTH and A-II stimulate adrenal cell steroid production, we investigated whether glucocorticoids could mediate the effects of these peptides on IGF-I receptors (Table 2). Pretreatment of adrenal cells for 48 h with 10^{-9}M or 10^{-6}M dexamethasone did not alter significantly (^{125}I) IGF-I binding. Cells were also cultured with 10^{-7}M A-II or 10^{-10}M ACTH in the presence of aminoglutethimide at a concentration which blocks by 90 % the stimulation of steroidogenesis induced by ACTH and A-II (4). Although aminoglutethimide alone decreases slightly (^{125}I) IGF-I binding, it has very small action on the stimulatory effects of A-II and ACTH on IGF-I receptors. Therefore, glucocorticoids do not seem to be the mediator of A-II and ACTH action on IGF-I receptors.

IGF-I, as many other polypeptidic hormones (4, 7), down-regulates its own receptors in several cell types (2, 8, 9) including adrenal cells. On the other hand, heterologous up-regulation of type I IGF receptors has been demonstrated in two other steroidogenic cells : Leydig cells and granulosa cells. In cultured pig Leydig cells, hCG produced a 2- to 3-fold increase of IGF-I receptors within 2 days (10).

Table 2. Effects of steroids on (^{125}I)IGF-I binding to bovine adrenal cells

Pretreatment	(^{125}I)IGF-I bound (% of control)		
	Control	A-II	ACTH
Control	100	170 ± 15	133 ± 13
Aminoglutethimide 1 mM	70 ± 6	145 ± 12	135 ± 6
Dexamethasone 10^{-9}M	92 ± 8		
10^{-6}M	104 ± 8		

Bovine adrenal cells were incubated for 48 h at 37°C with A-II (10^{-7}M), ACTH (10^{-10}M) or dexamethasone (10^{-9} or 10^{-6}M) in the absence or presence of aminoglutethimide (1 mM). After incubation, the medium was removed and replaced by binding medium. The results represent mean ± SD of three experiments, each done in triplicate.

Similarly, administration of hCG for 5 days to hypophysectomized rats enhances IGF-I receptors of Leydig cells, the effects of FSH and growth hormone being far less (11). Further, treatment of cultured rat granulosa cells with FSH enhances IGF-I receptors (12). Thus, it appears that in the steroidogenic cells IGF-I receptors are positively regulated by the specific peptidic hormones. Physiologically, the effect might be important, since in the three cell types, adrenal, Leydig and granulosa cells, IGF-I is required for the expression and the maintenance of each cell differentiated function (3, 10, 13) and in each cell type the specific peptidic hormones stimulate IGF-I secretion.

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