# PROLACTIN RECEPTION AND ITS EFFECT ON ACTH BINDING IN HUMAN AND GUINEA PIG ADRENOCORTICAL CELLS

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Experimental results indicating that prolactin (PL) may play an important role in the regulation of adrenocortical function have been published with increasing frequency in recent years [1, 5, 8]. Specific PL receptors have been found in the adrenals of experimental animals [7, 9, 12]. However, PL reception has been studied only on microsomal preparations, which makes assessment of the significance of this process difficult. PL reception by human adrenal glands has not yet been studied at all.

The mechanism of the effect of PL in the adrenal cortex is not clear. One probable way of action of PL may be modulation of the effects of ACTH. Changes in hormone reception constitute an effective mechanism of modulating action, which is frequently manifested in the effect of PL on other targets [6].

With these considerations in mind we set out to study PL reception in the human and guinea pig adrenal cortex, using both membrane preparations and isolated cells, and also to determine whether saturation of PL receptors may give rise to changes in ACTH binding.

## EXPERIMENTAL METHOD

Suspensions of adrenocortical cells from adult guinea pigs and man (tissue obtained at adrenalectomy for Cushing's disease or pheochromocytoma) were used. Minced cortical tissue was incubated in Eagle's medium with 0.5% bovine serum albumin (BSA), 10 mM HEPES, and 0.25 mg/ml of collagenase ("Fluka," 270 mU/mg) at 34°C for 40 min. The tissue was then carefully disaggregated by repeated passage through a plastic pipet. The suspension was filtered through nylon gauze. The procedure was repeated a further 3 times, with incubation for 20 min each time. Cells were sedimented from the filtrates (600 g, 10 min), pooled, washed with Eagle's medium, and suspended in the same medium containing 0.5% BSA and 10 mM HEPES. The viability of the cells (over 95%) was determined with trypan blue, followed by morphological verification under the microscope. In some experiments the cells were fractionated in a stepwise Percoll density gradient (0, 20, and 60% solution), according to the instructions provided by the firm of "Pharmacia." With this gradient it is possible to obtain a virtually pure fraction of adrenocorticocytes.

Microsomes were isolated by differential ultracentrifugation in 0.25 M sucrose, suspended in 25 mM Tris-HCl buffer, pH 7.4, containing 10 mM $MgCl_2$ and 0.1% BSA, and kept until analysis at —20°C.

PL and ACTH (Kaunas Endocrine Preparations Factory) were iodinated ($^{125}I$) by the chloramine method, purified by gel chromatography, their native state verified, and their specific radioactivity determined [3, 12]. Binding of $^{125}I$-PL and $^{125}I$-ACTH by cells and microsomes was studied by the appropriate methods [10-12]. The standard sample for determination of binding contained the labeled hormone ($10^5$ cpm), $10^5$-$10^6$ cells or 300 $\mu$g microsomal protein, in a final volume of 250 $\mu$l. Binding was analyzed by Scatchard's method with determination of the binding parameters graphically and by the appropriate program for the "Élektronika MK-61" calculator [2]. The significances of differences was determined by the Wilcoxon—Mann—Whitney nonparametric test.

## EXPERIMENTAL RESULTS

Isolated adrenocortical cells, both human and guinea pig, specifically bound PL, and this process is characterized by saturation and reaches equilibrium in the course of 60 min (Fig. 1).

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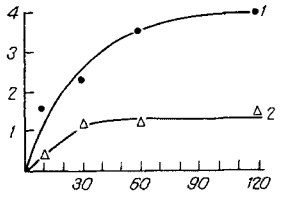


Fig. 1

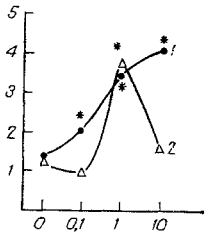


Fig. 2

Fig. 1. Dependence of $^{125}$I-PL binding by isolated guinea pig (1) and human (2) adrenocortical cells on time. abscissa, time, min; ordinate, $^{125}$I-PL specifically bound by cells, as a percentage of total amount.

Fig. 2. Effect of PL on binding of $^{125}$I-ACTH by isolated guinea pig (1) and human (2) adrenocortical cells. Abscissa, PL concentration in medium, μg/ml; ordinate, $^{125}$I-ACTH specifically bound by cells as a percentage of total amount of radioactivity. Asterisk indicates significant differences ($p < 0.05$) compared with control (0 μg/ml of PL). Each experiment was repeated 3 or 4 times.

The study of PL reception by the microsomal fraction of the adrenal cortex showed that it also can carry out intensive specific binding of the hormone. The binding parameters, determined by Scatchard's method, are evidence that cortical cell menbranes contain receptors with high affinity for PL: for instance, the association constant ($K_a$) for PL in microsomes of the guinea pig adrenal cortex varied between $0.14 \times 10^9$ and $0.20 \times 10^9$ $M^{-1}$ (n = 3), whereas the maximal binding capacity ($B_{max}$) was about $2 \times 10^{-10}$ M. These characteristics differed only a little from the binding parameters of PL by liver microsomes, which we determined for comparison ($K_a$ about $0.3 \times 10^9$ $M^{-1}$ and $B_{max}$ $1.1 \times 10^{-10}$ M). The study of isolated adrenocortical cells showed that the receptors exhibited even stronger affinity for the hormone than in microsomes: $K_a$ in this case reached $0.9 \times 10^9$ $M^{-1}$. In this case $B_{max}$ was lower (down to $0.3 \times M^{-10}$ M), evidently because in native cells a smaller fraction of the receptors is accessible for interaction with the hormone. Receptors of human adrenocortical microsomes possessed equal or stronger affinity for PL combined with smaller capacity: $K_a$ for microsomes of conventionally normal tissue from two patients undergoing operations for Cushing's disease was $3.0 \times 10^9$ and $0.7 \times 10^9$ $M^{-1}$, whereas $B_{max}$ was $1.2 \times 10^{-11}$ and $2.4 \times 10^{-11}$ M respectively. Both in man and in the guinea pig, PL binding sites of only one type were found, and in their parameters they correspond to the class of high-affinity receptors with limited binding capacity, features that were particularly distinct in the case of human adrenals.

The presence of specific high-affinity PL receptors in the guinea pig adrenals confirms results obtained by other workers who found that the parameters of PL reception by the adrenals are comparable in magnitude with those for other target tissues (mammary gland, liver, gonads) [7, 9, 12]. High-affinity PL receptors in the human adrenals are being characterized here for the first time. These data show that the adrenal cortex in man, as in other mammals, can exhibit sensitivity for PL which is not less than that of other targets of the hormone which have been studied, such as the mammary gland and gonads.

One way by which PL acts on its targets is by modulating their sensitivity to other hormones (Leydig cells, prostate gland, corpus luteum) [6]. It can therefore be postulated that a similar mechanism is utilized also in the adrenal cortex. The point of application for the action of PL in this case may be reception of ACTH, the principal regulator of corticosteroid production, which has received little study.

Intensification of binding of $^{125}$I-ACTH by guinea pig and human adrenocortical cells was observed 60 min after addition of PL to the medium (Fig. 2). The higher the PL concentration (within the range 0.1-10 μg/ml), the more ACTH was bound by the guinea pig's cells. Human cells were characterized by a marked maximum of binding, corresponding to a PL level in the medium of 1 μg/ml. Incidentally, the experiments with guinea pig cells were undertaken on an unpurified cell suspension, whereas the experiments with human cells were conducted both on an unpurified suspension and on adrenocorticocytes isolated on a stepwise Percoll density gradient (the results of a representative experiment on human cells isolated in a Percoll density gradient are given in Fig. 2).

Binding of ACTH and the effect of PL in a dose of 1 μg/ml on it was analyzed by Scatchard's method. Two classes of ACTH binding sites were found in the guinea pig: high-affinity with low binding capacity, and low-affinity with high capacity, as was found previously in experiments on membrane preparations [10]. Under the influence of PL

the concentration of low-affinity binding sites rose (from $6.4 \times 10^{-9}$ to $9.6 \times 10^{-9}$ M), whereas the remaining parameters of binding remained virtually unchanged. This suggests that intensification of ACTH binding by PL takes place on account of an increase in the number of accessible low-affinity and high-capacity receptors.

The mechanism of the modulating effect of PL on ACTH reception is not yet clear. It is unlikely that the biosynthesis of receptor molecules can be intensified by its action for 60 min. An increase in their number on the surface of the cytoplasmic membrane due to internalized receptors, possibly facilitated by corresponding changes in the lipid bilayer of the membrane, seems a more realistic explanation. In the rat liver, for example, PL increases membrane flowability and, at the same time, stimulates internal reception [4].

The investigation thus demonstrated the presence of specific high-affinity PL receptors in the human adrenal cortex, and this is confirmed for guinea pig adrenocortical tissue also. One way by which PL acts on corticosteroid production may be by the intensification of ACTH reception.

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