GLUCOCORTICOID BINDING TO PLASMA MEMBRANES OF THE ADENOHYPOPHYSIS

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Studies on glucocorticoid binding to the anterior pituitary gland has revealed, in addition to a receptor binding both natural and synthetic steroids, the presence of a compound closely resembling plasma transcortin (Koch, Lutz, Briaud & Mialhe, 1975, 1976; DeKloet & McEwen, 1976). Whether this transcortin-like component, which is not a true receptor and has been shown not to be due to mere blood contamination, is intracellular or bound to the cell membrane is still unclear. In this study, using isolated cells and plasma membrane fractions, we present indirect evidence suggesting attachment of the transcortin-like material to the cellular membrane of the pituitary cells.

Isolated pituitary cells were obtained as described by Portanova, Smith & Sayers (1970), using glands from 1-day adrenalectomized male rats, extensively perfused with ice-cold 0-9% saline through the heart. The washed cells were used to prepare a cytosol fraction in 10 mM-Tris-HCl (pH 7-2) containing 1 mM-EDTA, which was further heated at 40°C for 10 min. This treatment has been reported to inactivate the true receptor, while leaving intact the transcortin-like component. The cytosol was incubated at 0°C for 2 h with various amounts of [1,2-3H]corticosterone, obtained from the Radiochemical Centre, Amersham (sp.act. 112 Ci/mmole), in the presence or absence of a 1000-fold excess of unlabelled steroid. Separation of bound from unbound hormone was performed by Sephadex G-25 gel filtration. When the plasma membrane fraction was to be obtained, whole adenohypophyses were homogenized with the aid of a Dounce homogenizer in 50 mM-Tris-HCl (pH 7-3), supplemented with 1 mM-MgCl₂, 1 mM-dithiothreitol and 250 mM-sucrose. After centrifugation at 600 g for 10 min, the supernatant was centrifuged further at 10 000 g for 15 min and the pellet resuspended in the same buffer. Samples of the crude plasma membrane preparation (80 to 120 μg protein) were incubated with 10 pmol [3H]corticosterone or [1,2,4-3H]dexamethasone, obtained from NEN Chemicals (sp.act. 21 Ci/mmole), in the presence or absence of a 200-fold excess of non-radioactive steroids. After incubation at 37°C for 10 min and at 0°C for 30 min, 50 μl aliquots were processed as described by Rodbell, Krand, Pohl & Birbaumer (1971).

Figure 1 clearly shows that the cytosol derived from pituitary cells did exhibit a transcortin-like component, whose apparent dissociation constant was similar to the value (4-9 nmol/l) obtained using the cytosol from whole glands. The concentration of binding sites, however, was reduced in that case, probably as a result of the trypsin treatment of the pituitary glands. Thus, these observations strongly suggest that the transcortin-like material is not just confined in extracellular spaces, but is intrinsic to the cell and located within the cell and/or attached to the plasma membrane.

To investigate the latter hypothesis, the binding properties of the plasma membranes were examined and competition studies were carried out using both natural and synthetic labelled glucocorticoids, as well as various unlabelled steroids. Figure 1b reveals that non-radioactive corticosterone and progesterone competed to a significantly greater extent (P<0.01, Duncan’s multiple range test) than dexamethasone for corticosterone-binding sites. This correlates well with the known steroid-binding specificity of transcortin (Westphal, 1971).
Fig. 1. (a) Scatchard plot of \([\text{3H}]\)corticosterone binding to the transcortin-like component in cytosol from isolated pituitary cells. \(K_d\) = dissociation constant; \(n\) = concentration of sites.

(b) Glucocorticoid binding of pituitary plasma membrane fractions incubated in the presence of \([\text{3H}]\)corticosterone and without (circles) or with excess unlabelled corticosterone (squares), dexamethasone (triangles) and progesterone (circles). (c) Binding of plasma membrane fractions incubated with \([\text{3H}]\)dexamethasone under the same conditions as above. Radioactivity, counted with an efficiency of 58%, is the mean ± S.E.M. of 6 determinations. Each experiment was repeated three times with similar results.

Strikingly, however, dexamethasone, which does not bind to transcortin and to the transcortin-like component, was able to displace radioactive corticosterone significantly \((P<0.01)\). This finding might be explained by non-specific uptake of \([\text{3H}]\)dexamethasone, as suggested in Fig. 1c. An alternative possibility could be that, in addition to the transcortin-like compound, the true receptor (which binds both glucocorticoids) might partly adhere to the cellular membrane.

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REFERENCES