The utility of the established ACTH secreting mouse pituitary tumor cell line AtT20 for investigating early glucocorticoid inhibition was examined. Three different strains of the cell line D1, D16v, and D16:16, respectively, were analyzed. In initial studies CRF and phorbol esters were used as secretagogues to examine the properties of hormone secretion. In a perifusion system (cells in suspension) D1 cells failed to respond to the secretagogues, whereas both D16v and D16:16 cells were responsive. However, hormone release declined upon repeated exposure to secretagogue in both D16v and D16:16 cells and similar data were obtained when cells adhering to cover slips were perifused. In static incubation D16:16 cells gave more consistent results especially with respect to inhibition by glucocorticoids and were used in all subsequent studies. Synthetic glucocorticoids acting through the type II receptor inhibited CRF-induced ACTH release within 45 min; at 120 min, stimulated release was strongly (80-90%) suppressed. In contrast, no consistent inhibition by corticosterone could be found. In the presence of glycyrrhetinic acid, an inhibitor of 11 beta-hydroxysteroid dehydrogenase, a high concentration of corticosterone (10 microM) did produce a slight inhibition of ACTH release. Dexamethasone also inhibited ACTH release induced by the calcium channel activator compound (+)202-791. The accumulation of cAMP in response to CRF was not altered by dexamethasone. The inhibitory effect of synthetic glucocorticoids on ACTH release was prevented by blockers of messenger RNA (actinomycin D, dichlorobenzimidazole ribofuranoside) or protein (puromycin) biosynthesis, indicating the induction of new proteins. Immunoblotting for lipocortin I (annexin I) and chromogranin A revealed no induction by dexamethasone of any of these proteins in D16:16 cells. Messenger RNA encoding lipocortin I was not detectable and was not induced by treatment with dexamethasone in D16:16 cells. These data show that the AtT20 D16:16 strain is a useful model for early glucocorticoid action, which is mediated by type II receptors and involves the induction of new protein(s). Notably, induction of lipocortin I messenger RNA or protein could not be detected at a time when the inhibitory effect of glucocorticoids on stimulated hormone secretion was maximal [1)].

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Results demonstrated that triptolide inhibited cell viability and colony number of AtT20 cells in a dose- and time-dependent pattern. Triptolide also suppressed proopiomelanocortin (Pomc) mRNA expression and extracellular adrenocorticotropic hormone (ACTH) secretion in AtT20 cells. Flow cytometry prompted that triptolide leaded to G2/M phase arrest, apoptosis program and mitochondrial depolarization in AtT20 cells. Moreover, dose-dependent activation of caspase-3 and decreased Bcl2/Bax proportion were observed after triptolide treatment. By western blot analysis Li et al. found that triptolide impeded phosphorylation of NF-κB p65 subunit and extracellular signal-regulated kinase (ERK), along with reduction of cyclin D1, without any impact on other NF-κB related protein expression like total p65, p50, IκB-α, p-IκB-α. Furthermore, the mouse xenograft model revealed the inhibition of tumor growth and hormone secretion after triptolide administration. Altogether this compound might be a potential pharmaceutical choice in managing Cushing's disease [2)].

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2)
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