Posttranslational Modulation of Glucocorticoid Feedback Inhibition at the Pituitary Level

MIN CHIN LIM, MICHAEL J. SHIPSTON, AND FERENC A. ANTONI

Diagnostic tests of hypothalamic-pituitary-adrenocortical function in psychiatric illness largely report the interaction of hypothalamic secretagogues with glucocorticoids at the pituitary level. This study investigated whether the efficiency of glucocorticoid inhibition is subject to modulation by intracellular processes that enhance cAMP accumulation and/or facilitate membrane depolarization. The secretion of ACTH induced by corticotropin-releasing factor (CRF; 0.1 nM) in primary cultures of rat anterior pituitary cells was markedly inhibited upon a 2-h exposure to 100 nM corticosterone. Arginine vasopressin (2 nM) enhanced the cAMP as well as the ACTH responses to CRF and reduced the efficiency of glucocorticoid inhibition of ACTH release. The action of arginine vasopressin was mimicked by rolipram, an inhibitor of cyclic nucleotide phosphodiesterase type 4. Application of the broad specificity K+ channel blockers clofilium and astemizole produced minor or no significant enhancement of CRF-induced ACTH release, respectively, but opposed the inhibitory effect of corticosterone. Specific blockers of HERG, KCNQ, and Isk channels had no effect on ACTH release under any condition examined. In summary, these data reveal multiple sites of posttranslational modulation of adrenal corticosteroid action at the level of the pituitary gland, which appear important for the outcome of diagnostic tests of hypothalamic-pituitary-adrenocortical function. (Endocrinology 143: 3796–3801, 2002)
Materials and Methods

Reagents

Reagents for tissue culture and RIAs were previously described (23). Synthetic human 41-amino acid residue CRF and AVP were obtained from Bachem (St. Helens, UK). Astemizole was purchased from Sigma-Aldrich (Gillingham, UK). Clofilium tosylate was obtained from RBI (Sigma, Poole, UK). The following compounds were generously provided by the manufacturers: Rolipram—Schering AG (Berlin, Germany); E4031—Eisai Chemical Co. Ltd. (Kashima-gun, Ibaraki-ken, Japan); dofetilide (UK-68,798)—Pfizer Central Research (Sandwich, UK); WAY-123,398—Wyeth-Ayerst Research (Princeton, NJ); and chromanol 293B—Hoechst-Marion (Frankfurt/Main, Germany).

Cell cultures

Dispersed rat anterior pituitary cells were prepared from the pituitary glands of male Wistar rats (30–40 d postnatally; Charles River Ltd., Margate, UK) as previously described (23). Approximately 5 × 10⁴ cells/well were plated in 24-well tissue culture plates (Falcon, B&D, Le Point de Claix, France) and cultured in DMEM containing 2.5% fetal bovine serum (Harlan-Sera-lab, Belton, Loughborough, UK) and 7.5% horse serum (Sigma, H-1338). Monitoring of the ACTH response to various stimuli was as detailed previously (23). Briefly, corticosterone or vehicle was applied in fresh, serum-free medium 2 h before stimulation with CRF. Blockers of K⁺ channels were applied 30 min before and during the 60-min incubation with CRF. Blockers of K⁺ channels were applied 30 min before and during the 60-min incubation with CRF. In some experiments cellular cAMP content was also measured. The cells were pretreated as described for ACTH release, and the incubation was terminated by aspiration of the medium, immediately followed by the addition of 0.1 M HCl. Cellular cAMP was extracted into 0.1 M HCl by repeated freeze-thawing, acetylated, and quantified by RIA (24).

Data analysis

Data were evaluated by one- or two-way ANOVA, followed by multiple comparisons as appropriate. Results presented as a percentage of control ACTH release were derived from the raw data according to the formula: (X − B)/(C − B) × 100%, where X is the value analyzed, B is the basal ACTH secretion, and C is the amount of ACTH observed in the presence of a control stimulus.

Results

Vasopressin (2 nM) enhanced the response to CRF (0.1 nM), but failed to stimulate ACTH when given alone (Fig. 1A). Pretreatment with corticosterone produced a dose-dependent inhibition of CRF-induced ACTH release (Fig. 1B). The efficiency of this inhibition was markedly reduced by AVP. The cAMP phosphodiesterase inhibitor rolipram mimicked the effects of AVP on the ACTH response as well as its inhibition by corticosterone (Fig. 2). AVP enhanced the cAMP response to CRF at both 10 and 60 min (Fig. 3A). A series of similar studies with various CRF and AVP concentrations revealed that the ability of 100 nM corticosterone to suppress ACTH secretion markedly diminished as the agonist-evoked cAMP response increased (Fig. 3B).

Plasma membrane K⁺ channels are prominent targets of cAMP-dependent phosphorylation (25) and have been previously implicated in the action of glucocorticoids in corticotrope cells (8) as well as in hippocampal pyramidal neurons (26, 27). As previous results with the blocker tetraethylammonium (26) excluded the involvement of a number of well characterized K⁺ channels in glucocorticoid action in our experimental system, the class III antiarrhythmic agent clofilium and the antihistamine drug astemizole were tested. These drugs are blockers of HERG, KCNQ, and IsK type channels, all of which are expressed in pituitary cells.
Discussion

These data demonstrate that the agonist-induced cAMP response is a major determinant of the outcome of early gene induction by glucocorticoids. Furthermore, they raise the possibility that novel plasma membrane K^+ channels mediate corticosteroid negative feedback in anterior pituitary corticotrope cells. Collectively, the results highlight the potential for posttranslational modulation of the negative feedback action of adrenal corticosteroids at the pituitary level.

The idea that AVP reduces glucocorticoid feedback was previously proposed (see Ref. 18 for review). However, the issue has not been addressed directly using physiological levels of secretagogue in anterior pituitary corticotrope cells. Collectively, the results highlight the potential for posttranslational modulation of the negative feedback action of adrenal corticosteroids at the pituitary level.

The level of CRF in pituitary portal plasma is maximally 0.3 nm; at this concentration corticotropes are far from maximally stimulated. In vivo, further enhancement of the hypothalamic drive is achieved by AVP, which is copackaged and cosecreted with CRF in parvicellular hypophysiotropic neurons of the hypothalamic paraventricular nuclei (18, 30–32). In addition, there is evidence that AVP is released into hypophysial portal blood by the anatomically distinct vaso-
association of dexamethasone nonsuppression in humans with elevated plasma AVP, were listed in the introduction. Taken together, these studies have provided evidence that AVP counteracts glucocorticoid feedback in humans.

The principal mode of action of AVP in corticotroph cells is amplification of the cAMP response to CRF through protein kinase C (18, 39). The enhancement of the cAMP response to low physiological concentrations of CRF by AVP is dramatic, up to 10- to 15-fold in the physiological range, and is characterized by acute plastic changes in the cAMP signaling cascade (40, 41). The augmentation of CRF-induced ACTH release by AVP is modest by comparison with the enhancement of the cAMP response. Thus, it is reasonable to suggest that amplification of the CRF-evoked cAMP has downstream targets other than CRF-evoked exocytosis. Indeed, the present study shows directly that the efficiency of glucocorticoid feedback on stimulated ACTH release is inversely related to the secretagogue-induced cAMP response.

The finding that exaggerated cAMP accumulation and consequently augmented activation of protein kinase A can override glucocorticoid inhibition is in agreement with the hypothesis that glucocorticoid feedback action is mediated by a protein phosphatase that opposes the action of protein kinase A (42). Plasma membrane K⁺ channels are common targets of protein kinase A and glucocorticoid inhibition (8, 23). In AtT20 and transfected HEK293 cells, glucocorticoids modulate the activity of a protein phosphatase in the vicinity of the STREX variant BK channel to counteract protein kinase A stimulated by CRF-induced cAMP (42, 43). In rat anterior pituitary cells, depolarization of the membrane potential abbreviated glucocorticoid feedback inhibition of stimulated ACTH release (3, 23, 44), which is consonant with the hypothesis that feedback is mediated by plasma membrane K⁺ channels. However, no evidence for the involvement of BK or other tetraethylammonium-sensitive K⁺ channels could be demonstrated in cultured rat anterior pituitary cells (23). The findings with clofilium and astemizole presented here suggest that atypical, as yet uncharacterized plasma membrane K⁺ channels may mediate glucocorticoid feedback inhibition in cultured rat anterior pituitary corticotropes. Specific blockers of HERG-, KCNQ-, and Islk-type K⁺ channels, all of which are reportedly expressed in the adenohypophysis (28), failed to alter the effect of corticosterone. It is of note that the concentration of astemizole required to oppose glucocorticoid inhibition was similar to that required for the blockage of store-operated Ca²⁺ channels (SOCs) in GH₃ pituitary tumor cells (45). Intriguingly, dofetilide was inactive in this respect, similar to the present findings (45). It is not immediately obvious how SOCs would be important for early inhibition by glucocorticoids. However, Ca²⁺ entry via SOC could be intricately linked to subplasmalemmal changes in intracellular Ca²⁺ and could be important for the activity of Ca²⁺-activated K⁺ channels that mediate glucocorticoid feedback inhibition (26, 27, 46). Clofilium as well as astemizole inhibit more than one type of K⁺ channel (47-50). Thus, it cannot be excluded that their action to oppose glucocorticoid feedback is due to generalized depolarization, which is known to reduce the efficiency of glucocorticoid inhibition (3, 23).

In summary, these data show that the efficiency of corti-

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<tr>
<th>Clofilium (μM)</th>
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<tr>
<td>0</td>
<td>300</td>
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<tr>
<td>0.3</td>
<td>250</td>
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<td>1</td>
<td>200</td>
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* P < 0.05 compared with corresponding stimuli in the absence of clofilium or astemizole. Where error bars are not visible, the SEM is less than the space required for the symbol. Results shown are representative of three and two experiments for A and B, respectively.

Fig. 4. Clofilium and astemizole attenuate the inhibition of CRF-stimulated ACTH release by 100 nM corticosterone. The effects of clofilium (A) and astemizole (B) on basal or 0.1 nM CRF-evoked ACTH release in the absence (●) and presence (▲) of 100 nM corticosterone (CORT) pretreatment are shown. Data are expressed as a percentage of the net ACTH release evoked by the respective control stimulus. Values are the mean ± SEM (n = 3–7/group). *, P < 0.05 compared with corresponding stimuli in the absence of clofilium or astemizole.
corticosteroid feedback inhibition may be markedly modified by agents acting on the cellular targets of glucocorticoid-
induced proteins. A fundamental determinant of the effi-
ciency of feedback inhibition is the magnitude of the cAMP
response evoked by hypothalamic secretagogues. Finally,
whether the observed effects of clofunic and astemizole on
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Fig. 5. Efficiency of corticosterone feed-
back inhibition is reduced by astemizole and clofunic. A, ACTH response to 0.1
mM CRF and 10 μM astemizole (AST). Solid columns
are means; bars indicate the SEM (n = 3–5/group). * P < 0.05 compared
with baseline by one-way ANOVA, followed by Newman-Keuls test. B, Inhibition
of 0.1 mM CRF-evoked ACTH release by corticosterone in the absence and pres-
ence of 10 μM astemizole (n = 4–5/group). *, P < 0.05 compared with corresponding
CRF group by one-way ANOVA, followed by orthogonal contrasts. The results
shown are representative of four experiments. C, ACTH response to 0.1 mM CRF
and 10 μM clofunic (CLOF). Solid col-
umns are means; bars indicate the SEM
(n = 4–5/group). *, P < 0.05 compared with
CRF (by one-way ANOVA, followed by
Newman-Keuls test). D, Corticosterone
inhibition of 0.1 mM CRF-evoked ACTH release in the absence and presence of 10
μM clofunic (n = 4–5/group). *, P < 0.05 compared with the corresponding CRF
group by one-way ANOVA, followed by
orthogonal contrast of means. Results
shown are representative of three exper-
iments.

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