Rapid Non-Genomic Feedback Effects of Glucocorticoids on CRF-Induced ACTH Secretion in Rats

Burkhard Hinz[1,3] and Rolf Hirschelmann[2†]

Received June 5, 2000; accepted July 7, 2000

Purpose. The present study investigates fast negative feedback actions of corticosterone (corticosteroid type I/type II receptor agonist) and RU 28362 (corticosteroid type II receptor agonist) on corticotropin-releasing factor (CRF)-induced adrenocorticotropic hormone (ACTH) secretion in rats.

Methods. To induce fast feedback, glucocorticoids were administered intravenously immediately before injection of the hypophyseotropic stimulus CRF. Plasma ACTH levels, being determined 5 to 30 min thereafter, were used as markers of fast feedback.

Results. Fast inhibitory effects on CRF-induced ACTH secretion became evident within 15 min (corticosterone) and 5 min (RU 28362) after steroid administration. Rapid feedback inhibition was also observed in the presence of other corticosteroids (cortisol, dexamethasone, aldosterone), whereas structurally-unrelated steroids (β-estradiol, progesterone, potassium canrenoate, alphaxalone) were inactive in this respect. Pretreatment of rats with the corticosteroid type II receptor antagonist RU 486 or the transcription inhibitor actinomycin D left fast feedback effects unaltered.

Conclusions. Our results demonstrate that glucocorticoids exert fast negative feedback at the pituitary level via a mechanism that is independent of corticosteroid type II receptor occupation and de novo synthesis of mRNA. In conclusion, corticosteroid-specific non-genomic effects may underly rapid glucocorticoid responses on CRF-induced ACTH secretion.

KEY WORDS: fast feedback; pituitary; corticosterone; RU 28362; corticotropin-releasing factor; corticosteroid type II receptor; non-genomic glucocorticoid action.

INTRODUCTION

Glucocorticoids are known to exert the major part of their effects genomically, i.e. via occupation of cytosolic glucocorticoid receptors, translocation of the glucocorticoid-receptor-complex into the nucleus, and subsequent activation or repression of de novo synthesis of mRNA and protein (1–3). As a result of this large array of steps, genomic effects are characterized by a lag-period ranging from at least 30 min to several hours and even days between the entry of the glucocorticoid into the cell and the manifest action of its hormonal response. However, compelling evidence suggests that this traditional theory of steroid hormone action is not sufficient to explain the broad spectrum of glucocorticoid effects. In addition to genomic effects, glucocorticoids may exert non-genomic actions occurring instantenously after glucocorticoid exposure or with a very short latency (4–6).

The release of glucocorticoids by the hypothalamo-pituitary-adrenal axis (HPA axis) is determined by a dynamic regulatory system consisting of the circadian rhythm, the response to stress and a complex negative feedback system. Negative feedback actions of glucocorticoids on the HPA axis have been divided into three distinct time domains: (i) fast feedback, evident within seconds to minutes; (ii) intermediate (early delayed) feedback, manifest within two hours; and (iii) slow (late delayed) feedback, apparent after several hours of exposure to glucocorticoids (7). Fast feedback has been inplicated in the control of the degree and duration of stress-induced adrenocorticotropic hormone (ACTH) and corticosteroid increases (7). Fast negative feedback effects have been extensively studied in rats by injection of corticosteroids shortly before the onset of stress (8–11). However, while it is generally accepted that delayed feedback effects require occupation of intracellular glucocorticoid receptors as well as de novo synthesis of mRNA and protein (7), the issue how glucocorticoids mediate fast feedback is still subject of debate. Most investigations published so far have looked at the mechanism underlying fast feedback using in vitro models ranging from primary pituitary cell cultures (12–14), pituitary cell fragments (15) to cells of the pituitary tumor cell line AtT-20 (16) that make it difficult to predict the situation in vivo.

The present study investigates fast negative feedback actions of corticosterone (corticosteroid type I/type II receptor agonist) and RU 28362 (corticosteroid type II receptor agonist) on corticotropin-releasing factor (CRF)-induced ACTH secretion in vivo in rats. To induce fast feedback, glucocorticoids were administered intravenously immediately before injection of the hypophyseotropic stimulus CRF. Plasma ACTH levels, being determined 5 to 30 min thereafter, were used as markers of fast feedback. Here we show that rapid feedback actions occurring within 5 to 15 min after glucocorticoid administration, were not affected by the corticosteroid type II receptor antagonist RU 486 and the transcription inhibitor actinomycin D suggesting non-genomic effects as underlying mechanism.

MATERIALS AND METHODS

Animals

Female Wistar rats [Crf:(WI)BR], weighing 200–300 g, were housed in groups of three per cage with free access to standard food and tap water. Rats were maintained under standard light (lights on from 06.00 to 18.00 h) and temperature (23 ± 1 °C) conditions. Rats were handled repeatedly before the experiments. Experiments were started between 09.00 and 10.30 h.

Drugs

Actinomycin D, corticosterone, cortisol, β-estradiol, potassium canrenoate and progesterone were bought from

[1] Department of Experimental and Clinical Pharmacology and Toxicology, Friedrich Alexander University Erlangen-Nürnberg, Fahrstrasse 17, 91054 Erlangen, Germany.
[2] Department of Pharmacology and Toxicology, School of Pharmacy, Martin Luther University Halle-Wittenberg, Wolfgang-Langenbeck-Strasse 4, 06120 Halle (Saale), Germany.
[3] To whom correspondence should be addressed.(e-mail: hinz@pharmakologie.uni-erlangen.de)
† Deceased.

ABBREVIATIONS: ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor; HPA axis, hypothalamo-pituitary-adrenal axis; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous.

Sigma (Deisenhofen, Germany). Ampoules containing dexamethasone disodium phosphate were obtained from Merckle (Blaubeuren, Germany). CRF was purchased from Calbiochem-Novabiochem (Läufelfingen, Switzerland). Sodium pentobarbital was obtained from Synopharm GmbH (Barsbüttel, Germany). Alphaxalone was from RBI (Natick, USA). RU 28362 and RU 486 were generously supplied by Roussel Uclaf, Romainville, France. The following vehicles were used for administration of test substances: 0.9% (w/v) NaCl (actinomycin D, CRF, potassium canrenoate, sodium pentobarbital), 0.9% (w/v) NaCl/ethanol with a final ethanol concentraqtion of 2% (v/v) (aldosterone, corticosterone, cortisol, RU 28362) and 0.9% (w/v) NaCl/2-hydroxypropyl-β-cyclodextrin (β-estradiol, progesterone). Alphaxalone and RU 486 were administered as suspensions (3 drops Tween 80/10 ml 0.9% (w/v) NaCl).

Experimental Protocol

Rats were anaesthetized with sodium pentobarbital (50 mg/kg i.p.). No additional anaesthetic doses were administered during experiments. Corticosterone (0.8 mg/kg i.v.) or RU 28362 (0.1 mg/kg i.v.) or vehicle were given 45 min after administration of sodium pentobarbital. Challenge was performed 15 seconds later by i.v. administration of 0.82 μg/kg human/rat CRF (1 μg/kg CRF acetate). Control animals received the respective vehicle. For i.v. injection, all substances and vehicles were administered into the tail vein of anaesthetized animals. To study mechanisms underlying fast feedback, rats were treated with RU 486 (25 mg/kg s.c., administered 45 min prior to the respective glucocorticoid) or actinomycin D (1 mg/kg i.v., administered immediately before the respective glucocorticoid into the tail vein) or the respective vehicle. Blood was taken at the indicated times from the retroorbital vein plexus, collected into prechilled polypropylene tubes containing EDTA disodium salt (1 mg/ml blood), centrifuged (3000 g, 10 min, 4 °C), and the plasma was removed for determination of ACTH levels. From each rat blood was obtained once, i.e. different animals were used for time-course experiments.

Hormone Determination

Plasma ACTH levels were measured in duplicate using a commercially available radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, USA).

RESULTS

Intravenous administration of CRF was accompanied by a rapid increase in basal ACTH levels with peak values occurring 5 min after administration (Fig. 1). Injection of corticosterone (0.8 mg/kg i.v.) immediately before CRF caused a significant decrease in CRF-induced ACTH levels within 15 min and a maximal inhibition 30 min after steroid treatment (Fig. 1). By contrast, treatment of rats with corticosterone without subsequent administration of CRF left basal ACTH secretion unaltered (data not shown). When using the selective corticosteroid type II receptor agonist RU 28362 (0.1 mg/kg i.v.) a significant decrease in CRF-induced ACTH secretion was already observed 5 min after steroid administration with a maximal inhibition at 30 min post-stimulation (Fig. 2). Administration of RU 28362 alone without subsequent

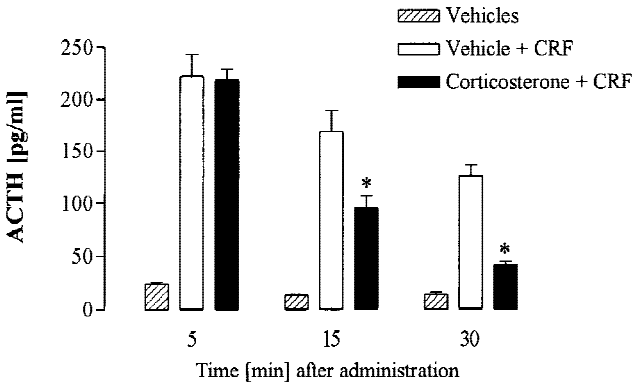

Fig. 1. Fast feedback effect of corticosterone (0.8 mg/kg i.v.) on CRF-induced ACTH secretion. Corticosterone or vehicle were administered 15 seconds before CRF (0.82 μg/kg i.v.) or vehicle. Blood sampling for determination of ACTH levels was performed 5, 15 and 30 min thereafter. Values are means ± SEM of n = 6–9 animals per group. *P <0.05 vs. corresponding CRF-treated group (Student's t-test).

CRF treatment did not alter basal ACTH levels in the time-course studied (data not shown). As shown in Fig. 3, the degree of fast feedback inhibition after administration of 0.1 mg/kg RU 28362 was significantly higher in comparison to the respective feedback effect exerted by the same dose of corticosterone. Rapid negative feedback effects were also observed in the presence of the corticosteroids cortisol, dexamethasone and aldosterone (Fig. 4). By contrast, structurally unrelated steroids such as β-estradiol, progesterone, potassium canrenoate and alphaxalone failed to inhibit CRF-induced ACTH secretion (Fig. 4).

To assess a possible involvement of corticosteroid type II receptors in fast feedback actions of the investigated glucocorticoids, further experiments were performed using the corticosteroid type II receptor antagonist RU 486. Pretreatment of rats with RU 486 failed to cause any significant change in rapid feedback effects of corticosterone and RU 28362 on CRF-induced ACTH secretion (Fig. 5). Fast feedback remained also unchanged when doubling the dose of RU 486 (50 mg/kg s.c.; data not shown). Administration of RU 486 without subsequent glucocorticoid treatment displayed no

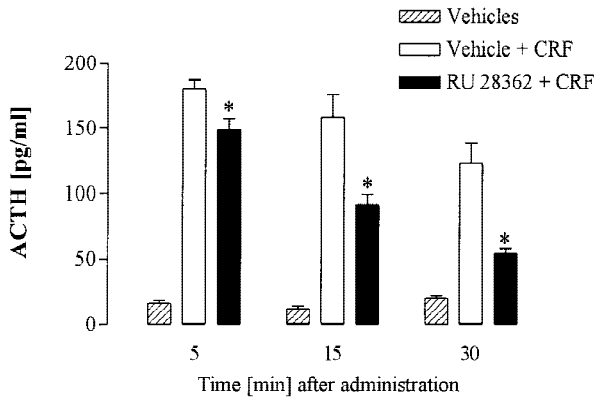

Fig. 2. Fast feedback effect of RU 28362 (0.1 mg/kg i.v.) on CRF-induced ACTH secretion. RU 28362 or vehicle were administered 15 seconds before CRF (0.82 μg/kg i.v.) or vehicle. Blood sampling for determination of ACTH levels was performed 5, 15 and 30 min thereafter. Values are means ± SEM of n = 7–8 animals per group. *P <0.05 vs. corresponding CRF-treated group (Student's t-test).

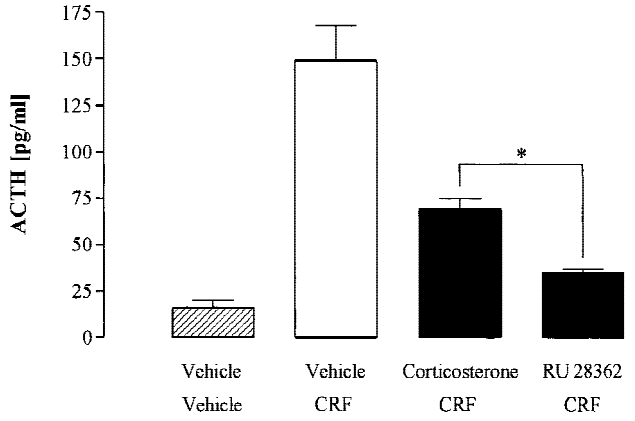

Fig. 3. Fast feedback effects of corticosterone (0.1 mg/kg i.v.) and RU 28362 (0.1 mg/kg i.v.) on CRF-induced ACTH secretion. Corticosterone or RU 28362 or vehicle were administered 15 seconds before CRF (0.82 μg/kg i.v.) or vehicle. Blood sampling for determination of ACTH levels was performed 30 min thereafter. Values are means ± SEM of n = 6–7 animals per group. *P <0.05 (Student's t-test).

significant influence on basal and CRF-induced ACTH secretion (Fig. 5).

To determine whether fast feedback effects require de novo synthesis of mRNA, the influence of the transcription inhibitor actinomycin D on fast glucocorticoid responses was investigated in further experiments. Treatment of rats with actinomycin D immediately before administration of the respective glucocorticoid did not interfere with fast negative feedback actions on CRF-induced ACTH secretion (Fig. 6). Under the same experimental conditions actinomycin D did not significantly alter basal as well as CRF-induced ACTH levels (Fig. 6).

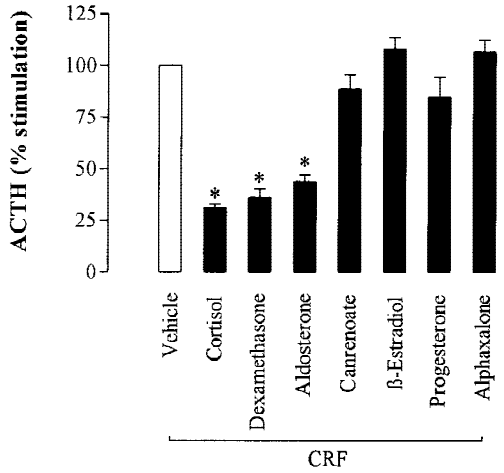

Fig. 4. Effect of cortisol (0.8 mg/kg i.v.), dexamethasone (0.1 mg/kg i.v.), aldosterone (0.8 mg/kg i.v.), potassium canrenoate (0.8 mg/kg i.v.), β-estradiol (0.8 mg/kg i.v.), progesterone (0.8 mg/kg i.v.) and alphaxalone (0.8 mg/kg i.p.) on CRF-induced ACTH secretion. Alphaxalone or vehicle were administered 1 min before CRF (0.82 μg/kg i.v.). All other steroids or its vehicles were administered 15 seconds before CRF (0.82 μg/kg i.v.). Dexamethasone was administered as the disodium phosphate salt at a dose equivalent to 0.1 mg/kg dexamethasone. Blood sampling for determination of ACTH levels was performed 30 min after steroid treatment. Values are means ± SEM of n = 6–8 animals per group. *P <0.05 vs. CRF-treated group (Student's t-test).

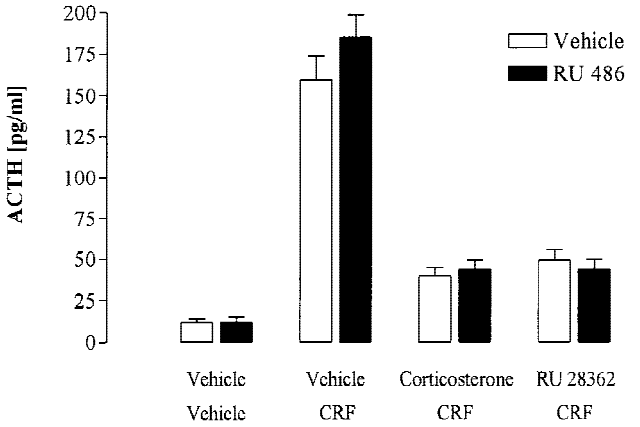

Fig. 5. Effect of RU 486 (25 mg/kg s.c.) on fast negative feedback effects of corticosterone and RU 28362 on CRF-induced ACTH secretion. RU 486 or vehicle were administered 45 min before corticosterone (0.8 mg/kg i.v.) or RU 28362 (0.1 mg/kg i.v.) or vehicle. Corticosterone or RU 28362 or its vehicle were injected 15 seconds before CRF (0.82 μg/kg i.v.) or vehicle. Blood sampling for determination of ACTH levels was performed 30 min thereafter. Values are means ± SEM of n = 6–9 animals per group.

DISCUSSION

Fast corticosteroid feedback has emerged as an important adaptive stress mechanism. Although fast feedback cannot prevent the onset of an initial response to a stressor, it might attenuate either degree and duration of the subsequent stress response (7). The physiological importance of rapid feedback response is also underlined by findings showing that the overactivity of the HPA axis, associated with depression (17) and chronic fatigue syndrome (18), may be attributable to a defective fast negative feedback system.

The present study demonstrates fast feedback actions of corticosterone and RU 28362 at the pituitary level in rats. Respective glucocorticoid effects fulfilled criteria typical for non-genomic steroid action in that they were rapid in onset and showed insensitivity to a competitive glucocorticoid receptor antagonist and an inhibitor of transcription.

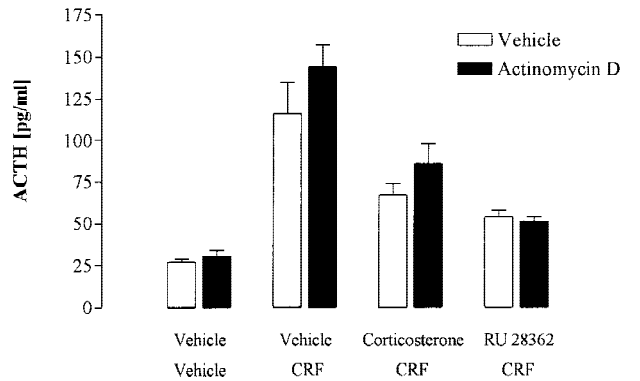

Fig. 6. Effect of actinomycin D (1 mg/kg i.v.) on fast feedback effects of corticosterone and RU 28362 on CRF-induced ACTH secretion. Actinomycin D or vehicle were administered immediately before corticosterone (0.8 mg/kg i.v.) or RU 28362 (0.1 mg/kg i.v.) or vehicle. Corticosterone or RU 28362 or its vehicle were injected 15 seconds before CRF (0.82 μg/kg i.v.) or vehicle. Blood sampling for determination of ACTH levels was performed 30 min thereafter. Values are means ± SEM of n = 6–9 animals per group.

Glucocorticoid-mediated rapid feedback effects occurred with lag-times of 5 min (RU 28362) and 15 min (corticosterone), respectively, that are incompatible with genomic steroid action, consisting of binding to cytosolic receptors, translocation of the steroid-hormone-complex into the nucleus, binding to DNA, and subsequent induction or repression of specific genes (1-3). In comparison to fast feedback exerted by corticosterone, the degree of rapid feedback inhibition caused by the same dose of the selective corticosteroid type II receptor agonist RU 28362 was higher. Moreover, RU 28362-mediated feedback inhibition was faster in onset already occurring 5 min after administration. Although we have not further addressed this issue in the present study, it is tempting to speculate that administration of RU 28362, which displays no affinity to transcortins (19,20), results in higher free concentrations of the steroid at its target site as compared to the transcortin-binding corticosterone. According to our results, rapid inhibitory effects of corticosterone and RU 28362 on ACTH secretion were confined to prior injection of the hypophyseotropic stimulus CRF and did not occur under basal conditions. These findings are in good line with data obtained in rat pituitary cells (12). On the other hand, fast feedback actions on basal ACTH secretion have been observed in humans following administration of corticosteroids (21,22).

A further major support for non-genomic events underlying fast feedback may be derived from our observation that rapid glucocorticoid effects were not affected by the glucocorticoid receptor antagonist RU 486 at a dose that was previously shown to be effective in antagonizing corticosteroid effects (23). Hence, occupation of intracellular glucocorticoid receptors is not involved in fast feedback responses of corticosterone and RU 28362. Previously, two corticosteroid receptor systems have been described in the rat brain. Glucocorticoid receptors, also referred to as type II corticosteroid receptors, have been demonstrated to become extensively occupied in the presence of the high corticosterone levels occurring during stress and circadian peak (20,23). By contrast, mineralocorticoid receptors (i.e., type I corticosteroid receptors) have been implicated in the control of ACTH and adrenal corticosteroid secretion at the low corticosterone levels observed during the circadian through in the activity of the HPA axis (20,23). Whereas type I corticosteroid receptors are predominantly localized in septo-hippocampal structures, type II receptors are widely distributed in brain, neurons and glia cells. Likewise, a high density of type II receptors has been demonstrated in the pituitary (20,23).

The involvement of non-genomic events in fast feedback actions was further substantiated by our finding that treatment of rats with the transcription inhibitor actinomycin D did not block rapid feedback effects. Remarkably, actinomycin D failed to suppress fast feedback effects at a dose that was previously shown to be effective in suppressing de novo synthesis of mRNA in rat pituitary (24) and dexamethasone-mediated delayed feedback in rats (25). According to our results, actinomycin D had no significant influence on basal as well as CRF-induced ACTH release suggesting that both events may represent secretion of a readily releasable pool and do not require de novo synthesis of mRNA.

Despite its first description more than fifty years ago (8), the question how glucocorticoids exert fast feedback is still a matter of debate. Accordingly, ambiguous in vitro results have been published with studies postulating either genomic (14) and non-genomic effects (12,13,16) as the underlying mechanism. In support of the data presented here, Widmaier and Dallman (12) as well as Abou-Samra et al. (13) were unable to block rapid glucocorticoid feedback responses with the translation inhibitor cycloheximide in perifused pituitary fragments and rat anterior pituitary cell cultures, respectively. However, the results of our in vivo study contradict the data published by Dayanithi and Antoni (14) which support the notion that fast negative feedback effects at the pituitary level are mediated by corticosteroid type II receptors and require de novo synthesis of mRNA and protein. Remarkably, in this investigation performed in perfused pituitary cell columns, fast feedback effects were observed with longer lag-times of 30 min (corticosterone) and 25 min (RU 28362), respectively. A possible site for non-genomic feedback inhibition may be second messenger pathways induced by ACTH secretagogues. In particular, in vitro glucocorticoids were shown to attenuate CRF-induced cyclic AMP generation and induction of inositol phosphate generation by arginine vasopressin (15). Interference with stimulus-induced second messenger generation might also explain our results showing that fast feedback is confined to stimulated ACTH secretion. Further mechanisms proposed for fast feedback include a direct antagonistic action of glucocorticoids on CRF binding to its pituitary receptor (26) or the release of molecular modified ACTH molecules with reduced biological activity (27).

Non-genomic effects of glucocorticoids occurring within seconds to minutes may be divided into specific non-genomic actions mediated by membrane-bound receptors and unspecific non-genomic events initiated by physico-chemical interactions with cellular membranes (5,6). However, in comparison to the concentrations necessary for specific non-genomic effects (i.e., $>10^{-9}$ M), higher glucocorticoid concentrations (i.e., 10^{-4} M) have to be used to provide direct interaction with biological membranes in vitro (4,6). With respect to the low corticosteroid doses used to induce fast feedback as well as its corticosteroid-specificity, it is conceivable that specific presumably membrane receptor-mediated non-genomic effects rather than non-specific physico-chemical interactions may underly rapid glucocorticoid effects described in this study. Previously, specific glucocorticoid binding sites have been identified on liver (28), neuronal (29) and pituitary membranes (30). However, if transcortin-like binding proteins identified on the surface of pituitary membranes (30) were responsible for rapid feedback effects, the non-transcortin-binding steroid RU 28362 (19,20) should be expected to leave CRF-induced ACTH secretion unaltered. Since RU 28362 indeed induced a substantial fast feedback inhibition, our data do not support hitherto identified corticosteroid pituitary binding sites as mediators of the observed glucocorticoid effects. However, a corticosteroid-specificity of rapid feedback actions may be derived from our data showing that fast feedback was confined to corticosteroids and was not elicited by structurally unrelated steroids such as β-estradiol, progesterone, potassium canrenoate and the steroid anaesthetic and 3α-hydroxy-pregnane derivative alphaxalone. This finding implies that specific corticosteroid structures confer the capacity to induce fast feedback. The possible involvement of putative corticosteroid-specific pituitary membrane receptors in fast feedback actions merits therefore further research.

In summary, our results show that rapid negative feed-

back effects of exogenous glucocorticoids on CRF-induced ACTH secretion in rats occur independently of glucocorticoid receptor occupation and de novo synthesis of mRNA. Thus, our data strengthens the notion that glucocorticoids mediate fast negative feedback via a non-genomic mechanism.

ACKNOWLEDGMENTS

The expert technical assistance of Petra Schwartz and Roswitha Wangemann is gratefully acknowledged.

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