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# The Adrenocorticotropin Stimulation Test: Contribution of a Physiologically Based Model Developed in Horse for Its Interpretation in Different Pathophysiological Situations Encountered in Man

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The present study aimed to characterize the adrenal response to ACTH. A model was developed that coupled the nonlinear disposition of cortisol with a physiologically based model for cortisol secretion by the adrenals. It was assumed that the response to ACTH resulted from two mechanisms: a stimulation of the cortisol secretion rate and control of the duration of the secretion. Seven dose levels of ACTH were tested in horses, a species similar to man as regards adrenal function. The main result was that the secretion rate of the adrenal gland can be modeled by a zero order process that is max-

imal for a relatively low dose of ACTH (0.1  $\mu\text{g}/\text{kg}$ ). Beyond this dose, the increasing adrenal gland response is only due to the prolongation of the time of its secretion. The consequences of these different features were explored by simulation to reproduce classical pathophysiological situations encountered in man. Our model was able to reproduce and simply explain many adrenal gland responses that are dimmed by the different nonlinearities of the system. (*Endocrinology* 147: 4281–4291, 2006)

ACTH STIMULATION TESTS are among the most commonly used methods for clinical assessment of the hypothalamic-pituitary-adrenal (HPA) axis. The iv injection of high doses (250  $\mu\text{g}$ ) of ACTH is a standard test for HPA evaluation (1, 2), but during the past decade, a low-dose (1  $\mu\text{g}$  or 0.5  $\mu\text{g}/\text{m}^2$ ) ACTH test has been advocated as a more sensitive method for the detection of certain impairments of adrenal function (3–5). Since then, a debate has arisen as to whether or not the low-dose ACTH test is more sensitive than the high-dose test in detecting mild degrees of adrenal insufficiency and on the place of rapid ACTH tests in comparison with insulin and metyrapone tests (6–8).

The interpretation of rapid ACTH stimulation tests is currently based on the measurement of serum or plasma concentrations of total cortisol before and 30 and 60 min after iv injection of ACTH (2). In fact, the measurement of total plasma cortisol concentration is only a surrogate biomarker that imperfectly represents the function of interest, namely the time profile of adrenal gland response in terms of cortisol secretion rate. Using the total plasma cortisol concentration

as an index of adrenal gland function may in fact lead to difficulties due to the nonlinear relationship existing between the measured total plasma cortisol concentration and the actual adrenal gland secretion rate.

The total cortisol concentration in blood is controlled not only by cortisol production (CP) rate (the parameter of interest) but also by processes of cortisol elimination and transport, the saturable binding to the corticosteroid-binding globulin (CBG), and a given total plasma cortisol concentration may correspond to different combinations of its three determinants. This is the case when the CBG level is altered in response to severe stress (9, 10).

To limit this first difficulty, we have developed a modeling approach to directly assess *in vivo* the cortisol binding parameters and the disposition of circulating cortisol not bound to CBG from the total plasma cortisol concentrations (11).

The second difficulty in interpreting rapid ACTH tests is that no more than two or three snapshot measurements of plasma cortisol are generally used as indicators of an overall adrenal response (AR). Little information is available to justify the choice of the standard sampling times used in these tests. More importantly, the factors governing the dose-response curve of the stimulatory effect of ACTH on circulating cortisol are poorly understood, and it will become apparent that peak cortisol concentration or area under the curve of cortisol *vs.* time profiles cannot be used indiscriminately as indicators of the adrenal gland response (12, 13) because they are not exploring the same underlying mechanisms.

A rational choice of cortisol sampling times may be documented from a mechanism-based model of the time devel-

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Abbreviations: AR, Adrenal response;  $\text{AUC}_{0-\text{last}}$ , area under the plasma concentration *vs.* time curve of total or CBG-free cortisol from time zero to the last concentration measured;  $B_{\text{max}}$ , CBG maximal binding capacity; CBG, corticosteroid-binding globulin;  $\text{Cl}_{\text{CBG-free}}$ , plasma clearance of CBG-free cortisol;  $\text{CBG}_{\text{free}}$ , plasma CBG-free cortisol concentration;  $C_{\text{max}}$ , plasma peak; CP, cortisol production; HPA, hypothalamic-pituitary-adrenal; RT, releasing time.

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opment of cortisol secretion in response to iv ACTH. Because intraadrenal cortisol storage is minimal, the acute action of ACTH on the adrenal cortex is mainly to increase cortisol synthesis (14) and only secondarily to increase cortisol secretion. It has been shown that the  $EC_{50}$  of ACTH(1–24) is in the picomolar range for CP in human adrenocortical cells (15) or the stimulation of cAMP production with cloned mouse ACTH receptors (16). On the other hand, peak plasma ACTH concentrations in humans have been reported to be around 1,900 and 66,000 ng/ml after low- and high-dose ACTH tests, respectively (6), corresponding to 633 and 22,000 pM concentrations. Comparison of these *in vitro* and *in vivo* data suggests that the ACTH concentrations obtained just after ACTH administration are able to maximally stimulate the ACTH receptors in the adrenals, thereby ensuring a maximal response in terms of cortisol secretion. In this context, the duration of maximal stimulation of the adrenal gland will depend mainly on the decay of the plasma ACTH concentrations, *i.e.* on the plasma ACTH half-life and not on the adrenal gland responsiveness.

In the present study, we aimed to characterize the time development of the AR to increased doses of iv administered ACTH, using horses as an experimental model because of the similarities of their HPA axis function to that of humans, in terms of cortisol plasma levels, circadian rhythm, and production rate (17, 18). Simultaneous recourse to a model for the disposition of cortisol not bound to CBG and a physiologically based model for cortisol secretion by the adrenals enabled us to explore the overall plasma cortisol *vs.* time profile in response to iv ACTH and to simulate different what-if pathophysiological scenarios.

## Materials and Methods

### Animals

Eight healthy saddle-bred horses (~9 yr old) weighing  $512 \pm 29$  kg were used. The horses were weighed three times during the course of the experiments with a 2-month interval between each weighing; the coefficients of variation were less than 4%. The horses were exercised 1–2 h/d, housed in individual boxes, and fed an appropriate commercial diet of two meals per day, with straw and water given *ad libitum*. They were accustomed to the sampling procedures and did not show any sign of agitation or stress.

All procedures involving animals were performed in accordance with the French legal requirements regarding the protection of laboratory animals and with the Authorization for Animal Experimentation no. 001889 of the French Ministry of Agriculture.

### Experimental design, administrations, and blood sampling

Total and CBG-free plasma cortisol concentration *vs.* time profiles were obtained after administration of seven doses of synthetic peptide ACTH(1–24) (Synacthène immédiat, Novartis, Basel, Switzerland; 0.25 mg/ml). The experiment was carried out with an eight  $\times$  eight crossover design, each horse receiving a placebo (0.9% NaCl) and seven ACTH doses (0.005, 0.01, 0.1, 0.5, 1, 2, and 10  $\mu$ g/kg). The time between consecutive ACTH administrations was about 4–7 d. Two horses were withdrawn during the study for intercurrent diseases not related to ACTH administration (colic).

Twelve and 2 h before the administration of ACTH, dexamethasone (Cortamethasone, Vetoquinol S.A., Magny-Vernois, France) was administered im at 0.1 mg/kg (50 ml for a horse of 500 kg) to suppress the basal secretion of cortisol.

ACTH was injected into the right jugular vein at 0900 h via an indwelling catheter inserted 5 min before administration. The catheter was rinsed with 2 ml of 0.9% NaCl after the ACTH injection. Blood (10

ml) was drawn by direct venipuncture (22-gauge needle) from the left jugular vein at –40 –20, 5, 10, 20, 30, 40, 50, 60, 90, and 120 min and each hour until 4 h (0.005  $\mu$ g/kg ACTH dose) or 8 h (0.01, 0.1, 0.5, 1, and 2  $\mu$ g/kg ACTH doses), and at –40, –20, 5, 15, 30, 60, 90, and 120 min and each hour until 8 h after the 10  $\mu$ g/kg ACTH dose.

Blood samples were collected in heparinized tubes and centrifuged at  $1400 \times g$  at 4 C for 10 min within 2 h of collection. The plasma was separated and aliquoted in three fractions and stored at –20 C until assay.

### Total cortisol assay

Cortisol was assayed by RIA (cortisol assay kit, Beckman Coulter, Roissy, France) in duplicate using 100- $\mu$ l aliquots of plasma. The calibration curve (10–300 ng/ml) was obtained with horse plasma stripped of steroids by charcoal. The level of quantification of the assay was 10 ng/ml. Within- and between-day coefficients of variation were lower than 15%.

### Data analysis

**Cortisol kinetic and binding parameters.** The pharmacokinetic part of the pharmacokinetic/pharmacodynamic model developed in this study is described by the set of equations presented in the supplemental data (published on The Endocrine Society's Journals Online web site at <http://endo.endojournals.org>). Briefly, this pharmacokinetic model describes the behavior of the total and free fractions of an analyte exhibiting nonlinear binding to plasma proteins. Such a model was first developed by Toutain *et al.* (19) for angiotensin-converting enzyme inhibitors and adapted for cortisol disposition by Picard-Hagen *et al.* (11).

This model incorporates the main kinetic and binding parameters for cortisol not bound to CBG (CBG-free cortisol) controlling circulating cortisol profiles: plasma clearance of CBG-free cortisol ( $Cl_{CBG-free}$ ), CBG maximal binding capacity ( $B_{max}$ ), and  $K_d$  CBG-free, equilibrium dissociation constant for CBG-free cortisol binding on CBG. The numerical values of these parameters had been determined during a previous experiment performed on the same horses. Briefly, this experiment consisted of the iv administration of cortisol at four dose levels (0.125, 0.25, 0.5, and 1 mg/kg). The four individual plasma cortisol concentration profiles obtained for each horse were simultaneously fitted using the set of equations presented in the supplemental data.

The mean values of  $Cl_{CBG-free}$ ,  $B_{max}$ , and  $K_d$  CBG-free are presented in Table 1. The binding parameters were used to calculate the plasma CBG-free cortisol concentrations ( $CBG_{free}$ ) from the total plasma cortisol concentrations (CORtot) using the following quadratic equation derived by Tait and Burnstein (20):

$$CBG_{free} = [(CORtot - B_{max} - K_d \text{ CBG-free}) + \sqrt{(K_d \text{ CBG-free} - CORtot + B_{max})^2 + 4 \times CORtot \times K_d \text{ CBG-free}}] / 2 \quad (1)$$

**AR to ACTH stimulation.** Different indices were used to describe the AR to increasing doses of ACTH: plasma peak ( $C_{max}$ ) of total or CBG-free cortisol, area under the plasma concentration *vs.* time curve of total or CBG-free cortisol from time zero to the last concentration measured ( $AUC_{0-clast}$ ), and CP (micrograms per kilogram). The  $C_{max}$  values of total and CBG-free cortisol were obtained by visual inspection of the plasma profiles of both fractions. The  $AUC_{0-clast}$  of total and CBG-free cortisol were calculated using the arithmetic trapezoidal rule. The CP over a specific time interval was calculated using the following equation:

**TABLE 1.** Mean pharmacokinetic and CBG binding parameters of CBG-free cortisol in eight horses

Parameters (units)	Mean $\pm$ SD
$Cl_{CBG-free}$ [ml/(kg·h)]	660 $\pm$ 71
$B_{max}$ nM ng/ml	204 $\pm$ 18 73.9 $\pm$ 6.52
$K_d$ CBG-free nM ng/ml	11.5 $\pm$ 5.3 4.17 $\pm$ 1.92

$$CP = AUC_{0\text{-clast CBG-free}} \times Cl_{\text{CBG-free}} \quad (2)$$

The ACTH-cortisol dose-response curves were fitted using a sigmoid  $E_{\text{max}}$  model, the so-called Hill equation, which is the Michaelis-Menten equation extended by a power coefficient and is traditionally used for sigmoid curve fitting (21):

$$AR = \frac{R_{\text{max}} \times D^n}{ED_{50}^n + D^n} \quad (3)$$

where  $AR$  is as previously defined,  $D$  is the dose of ACTH,  $R_{\text{max}}$  is the maximal  $AR$ ,  $ED_{50}$  is the dose of ACTH producing the half-maximum response, a parameter expressing ACTH potency, and  $n$  is the power coefficient, which describes the slope of the dose-effect curves and is a measure of sensitivity of the dose-effect relationship.

For each  $AR$ , the  $R_{\text{max}}$ ,  $ED_{50}$ , and  $n$  values were estimated by simultaneous fitting of individual dose-response curves using nonlinear least squares regression (Scientist, MicroMath Software, Salt Lake City, UT).

**Cortisol secretion process.** The cortisol secretion by adrenals after iv injection of ACTH was modeled using a physiologically based model taking into account the nonlinear disposition of cortisol (equations 1 and 2 in the supplemental data) and for which the cortisol input rate (cortisol secretion) was modeled as a zero order event ( $k_0$ ; micrograms per kilogram per minute) over releasing time ( $RT$ ; minutes). These parameters ( $k_0$ ,  $RT$ ) are linked to  $CP$  by the following equation:

$$CP = k_0 \times RT \quad (4)$$

Estimates of the secretion rate,  $k_0$ , and duration of secretion,  $RT$ , were obtained at each ACTH dose level by simultaneous fitting of the total plasma cortisol concentration profiles obtained for all the horses.

The increasing response of the adrenals when the ACTH doses increased was modeled by assuming that  $k_0$  and  $RT$  were dependent on the ACTH dose. The curves resulting from the relationships between  $k_0$  and  $RT$  estimates (dependent variables) and ACTH doses (independent variable) were fitted using the following equations:

$$k_0 = \frac{R_{\text{max}} \times D^n}{ED_{50}^n + D^n + \frac{(D^n)^2}{I^n}} \quad (5)$$

$$RT = \frac{R_{\text{max}} \times D^n}{ED_{50}^n + D^n} \quad (6)$$

where  $k_0$ ,  $RT$ ,  $D$ ,  $R_{\text{max}}$ ,  $ED_{50}$ , and  $n$  are as previously defined, and  $I$  is a factor with the dimension of a dose. Equation 5 was empirically selected to take into account the tendency toward a decrease in adrenal responsiveness to higher ACTH doses; the term:

$$\frac{(D^n)^2}{I^n}$$

is classically used in modified Michaelis-Menten equations describing enzymatic kinetics that exhibit inhibition by substrate excess (22).

Finally, plasma cortisol concentration profiles corresponding to different ACTH doses were simulated with a pharmacokinetic-pharmacodynamic model combining the pharmacokinetic disposition model for circulating CBG-free cortisol (see supplemental data) and the pharmacodynamic model describing the relations between ACTH doses and the cortisol secretion process (equations 5 and 6).

## Results

### Plasma cortisol profiles in response to ACTH stimulation

In all experiments, dexamethasone administration was followed by suppression of spontaneous cortisol secretion (total cortisol concentrations were below the quantification limits of the assay). The placebo (0.9% NaCl) did not elicit any quantifiable increase in total cortisol levels, indicating that the experimental procedures (*e.g.* placing catheters, giving

injections, collecting samples, etc.) were without measurable stimulatory effect on the HPA axis.

The plasma concentration *vs.* time profiles of total cortisol after iv administration of increasing doses of ACTH indicated a clear dose-response relationship (Fig. 1, A and B). The profiles corresponding to CBG-free cortisol (Fig. 1, C and D) were obtained from equation 1.

### ARs to ACTH stimulation

The relations between the ACTH doses and the five indices used to describe  $AR$ , namely total and CBG-free  $C_{\text{max}}$ , total and CBG-free  $AUC_{0\text{-clast}}$  and  $CP$ , are presented in Fig. 2. These dose-response curves were fitted using a sigmoid model (equation 3). Fitted curves and the corresponding estimated parameters are shown in Fig. 2.

The values of  $ED_{50}$  (ACTH potency) exhibited considerable differences when the adrenal gland response was assessed either in terms of  $C_{\text{max}}$  or overall plasma cortisol exposure ( $AUC_{0\text{-clast}}$ ), namely 0.012 and 0.054  $\mu\text{g}/\text{kg}$  for total and CBG-free  $C_{\text{max}}$  compared with 0.14 and 0.30 for total and CBG-free  $AUC_{0\text{-clast}}$ . In practice, this means that the  $C_{\text{max}}$  dose-response curve is shifted to the left compared with the  $AUC_{0\text{-clast}}$  dose response and that  $C_{\text{max}}$  reaches a maximal value at ACTH doses much lower than those required to obtain a maximal value for  $AUC_{0\text{-clast}}$  (Fig. 2).

$AUC_{0\text{-clast CBG-free}}$  and  $CP$  responses exhibited similar values for  $ED_{50}$  and  $n$ , as expected inasmuch as they are linked by a constant proportionality factor (equation 2).

### Modeling the cortisol secretion process

Two models, assuming either a first or zero order process for cortisol release by the adrenals, were investigated to analyze simultaneously the different profiles of total cortisol obtained with each dose of ACTH. Visual inspection of fitting revealed obvious misfits with the model that assumed a first order secretion process (data not shown) and goodness-of-fit criteria confirmed a better fitting of the model that assumed a zero order adrenal secretion process.

The fitted total plasma cortisol profiles of all the horses for the seven ACTH doses are presented in Fig. 3. Estimated values of  $k_0$  (secretion rate) and  $RT$  (duration of secretion), and the corresponding  $CP$  (equation 4) were obtained from these fittings and are presented in Table 2. Both  $k_0$  and  $RT$  exhibited an obvious tendency to increase with increasing ACTH doses (Table 2), with  $k_0$  tending to plateau at ACTH doses higher than 0.1  $\mu\text{g}/\text{kg}$ , whereas  $RT$  continued to increase over the range of ACTH doses tested.

The highest  $CP$  value calculated from equation 4, assuming a zero order secretion process for cortisol (136  $\mu\text{g}/\text{kg}$ , Table 2), was close to the maximal  $CP$  value estimated with equation 3 (123  $\mu\text{g}/\text{kg}$ , Fig. 2).

### Relations between cortisol secretion parameters and ACTH doses

Both parameters of cortisol secretion ( $k_0$  and  $RT$ ) were clearly related to the ACTH doses (Fig. 4). The  $k_0$  and  $RT$  *vs.* ACTH dose curves were best described by equations 5 and 6, respectively. Figure 4 shows the fitted curves and the corresponding parameters.

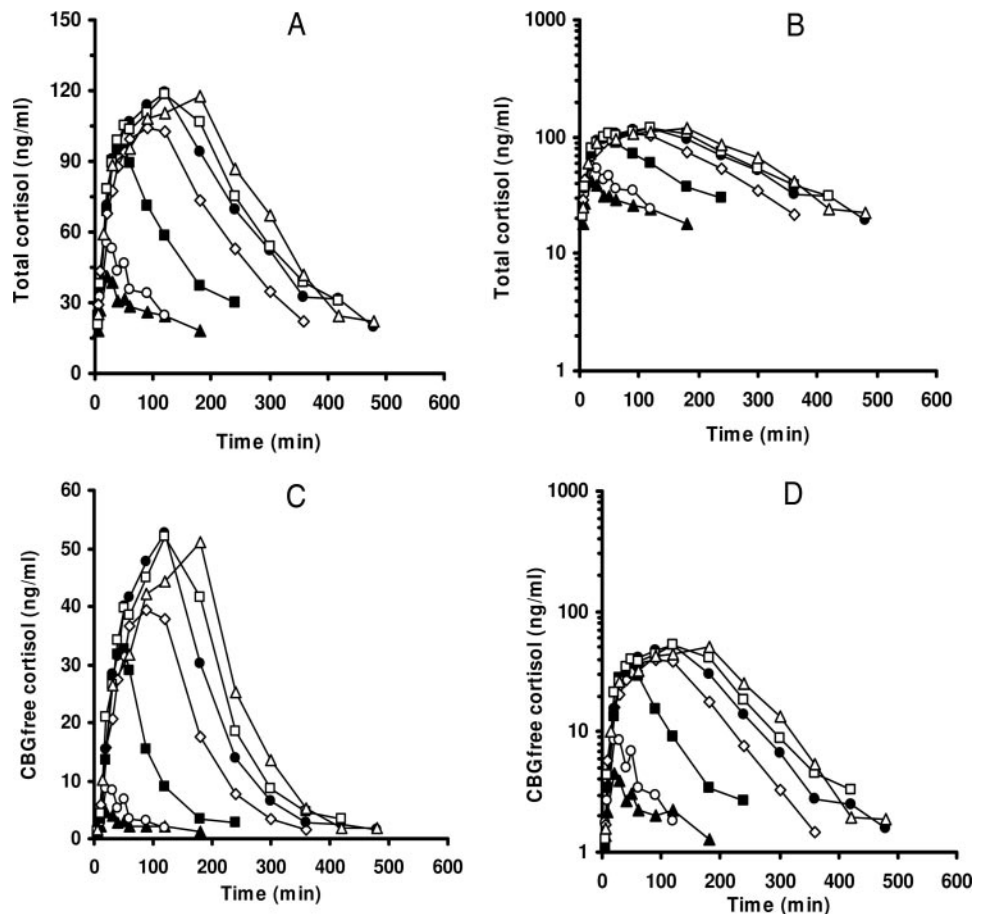


FIG. 1. Mean plasma cortisol concentrations after iv administration of ACTH in six to eight horses. The doses tested were: 0.005 (▲), 0.01 (○), 0.1 (■), 0.5 (◇), 1 (●), 2 (□), and 10 (△)  $\mu\text{g}/\text{kg}$ . Arithmetic (A and C) and semilogarithmic (B and D) plots represent total (A and B) and CBG-free (C and D) plasma cortisol concentration profiles. Visual inspection indicates for the ACTH dose of 0.1  $\mu\text{g}/\text{kg}$  and beyond that the secretion rate of the adrenal gland plateaued and that higher ACTH doses only increased the duration of the adrenal gland secretion.

Inspection of Fig. 4 clearly indicated that  $k_0$  reached a plateau for low ACTH doses, whereas RT continued to increase over the range of ACTH doses tested. Considering the 0.1–10  $\mu\text{g}/\text{kg}$  range of ACTH doses, the relations between  $k_0$  or RT and the ACTH dose can be summarized as an average maximal value of 0.91  $\mu\text{g}/(\text{kg}\cdot\text{min})$  for  $k_0$  (Fig. 4A, *dashed line*) and a log-linear relationship for RT (Fig. 4B, *dashed line*).

#### Pharmacokinetic/pharmacodynamic model linking ACTH doses to plasma cortisol profiles

By coupling the pharmacodynamic model connecting ACTH doses to the cortisol secretion process ( $k_0$ , RT) with the pharmacokinetic disposition model involving cortisol binding to CBG, it was possible to simulate total and CBG-free plasma cortisol concentration *vs.* time profiles for any ACTH dose from 0.005–10  $\mu\text{g}/\text{kg}$ .

Figure 5 shows the agreement between simulated CBG-free cortisol profiles and the corresponding average profiles obtained from experimental data over the ACTH range tested.

#### Simulations

By modifying some parameters from the cortisol secretion process, the disposition of circulating cortisol or both factors, we were able to simulate the AR to ACTH under several pathophysiological situations. Simulations were performed using three ACTH doses: 0.014, 0.05, and 5  $\mu\text{g}/\text{kg}$ . We se-

lected these ACTH doses to be relevant for comparisons with the doses used in humans for low- and high-dose ACTH tests. Indeed, in humans weighing 50–70 kg, the standard high ACTH dose (250  $\mu\text{g}$ ) corresponds to 3–5  $\mu\text{g}/\text{kg}$  and the standard low dose (1  $\mu\text{g}$ ) to 0.01–0.02  $\mu\text{g}/\text{kg}$ . So, in horses, the 0.014  $\mu\text{g}/\text{kg}$  dose is in the range of the low-dose ACTH test in humans, and the 5  $\mu\text{g}/\text{kg}$  dose corresponds to the high-dose ACTH test (the 0.05  $\mu\text{g}/\text{kg}$  dose is intermediate).

We first performed simulations corresponding to HPA axis stimulation with the three doses of ACTH (Fig. 6). Although differences in total CP were obvious between doses, the total cortisol concentration at 30 min post-ACTH was similar for the two highest doses.

Secondly, we performed simulations mimicking an adrenal insufficiency, corresponding to the halving of CP in response to iv ACTH. Figure 7 compares the total cortisol plasma profiles obtained by either halving the RT or  $k_0$  of the control condition, using ACTH doses 0.05 and 5  $\mu\text{g}/\text{kg}$ . Adrenal insufficiency simulated by decreased  $k_0$  led to diminished plasma cortisol responses at 30 and 60 min with both ACTH doses. The same was true for adrenal insufficiency due to decreased RT but only for the lower ACTH dose. In the simulation corresponding to reduced RT, plasma cortisol concentrations 30 and 60 min after the higher ACTH dose did not differ from normal, although sampling at 120 min allowed a clear-cut discrimination between normal and deficient states.

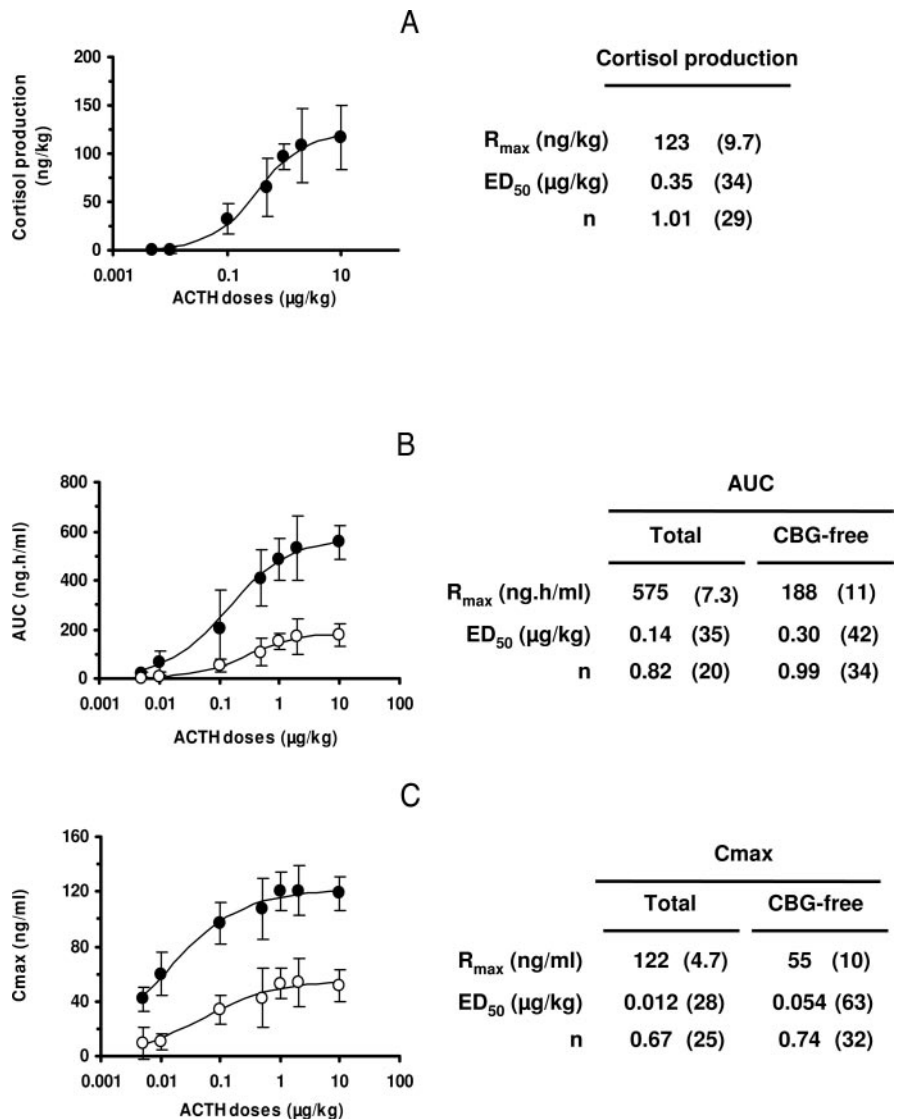


FIG. 2. Dose-response relationship between ACTH doses (0.005–10 mg/kg) and AR in terms of CP (A), AUC of plasma cortisol profiles (B), and peak plasma cortisol (C). ●, Total cortisol; ○, CBG-free cortisol. Experimental data (mean  $\pm$  SD) and fitted curves (—). Inspection of the figure indicates a lack of parallelism between total and CBG-free cortisol when the AUC is selected as an endpoint (B), whereas the responses are roughly parallel for total cortisol and CBG-free cortisol when the maximal plasma concentration is considered (C). Parameters of the dose-response relationship (equation 3) are presented:  $R_{\max}$ , maximal response;  $ED_{50}$ , ACTH dose producing half of the maximal response;  $n$ , Hill coefficient. Values shown are mean estimates (coefficients of variation, percentage).

In a final set of simulations, we investigated the effects on plasma cortisol profiles after ACTH administration (5  $\mu$ g/kg) of modifications of plasma cortisol binding, *i.e.* a modification of  $B_{\max}$ , resulting from variations in CBG concentrations. Firstly, changing the binding capacities induced obvious alterations in the concentration profiles of total cortisol (Fig. 8A), whereas there was no effect on the cortisol not bound to CBG (Fig. 8B). Secondly, we simulated simultaneous modifications of adrenal function (2-fold increase of the secretion rate,  $k_0$ ) and plasma cortisol binding (halving of CBG level  $B_{\max}$ ), a situation corresponding to chronic stress in horses and in humans (see *Discussion*). Comparison of the simulated cortisol profiles in normal and impaired situations showed that the decreased CBG concentrations compensated for the increased CP rate, such that the resulting total cortisol profiles did not differ sufficiently to permit discrimination at 30 and 60 min after ACTH (Fig. 8C). However, the CBG-free cortisol profiles after 5  $\mu$ g/kg ACTH differed in a manner that permitted discrimination between the two situations at the 30- and 60-min sampling times (Fig. 8D).

## Discussion

The main result of the present experiment was the 200-fold difference between the potency of ACTH to increase the two proposed mechanisms of the overall adrenal gland response to an ACTH treatment, namely  $k_0$  and RT of the cortisol secretion rate. ACTH potency as measured by the  $ED_{50}$  was 0.00347 and 0.72  $\mu$ g/kg for  $k_0$  and RT, respectively. Thus, the increased CP in response to increasing ACTH doses within the tested ACTH dose range (0.005–10  $\mu$ g/kg) was mainly due to the increase of RT, whereas  $k_0$  reached a maximal value at very low ACTH doses (0.1  $\mu$ g/kg). In other words, the ACTH tests are only able to explore concentration-dependent mechanisms of the adrenal gland response at very low ACTH doses but then reflect different time-dependent mechanisms as soon as the ACTH dose exceeds 0.1  $\mu$ g/kg.

In the present study, we aimed to build a physiologically based model describing the relation between an iv bolus of ACTH and AR in terms of plasma cortisol concentration *vs.* time profiles. The genuine feature of our model was to in-

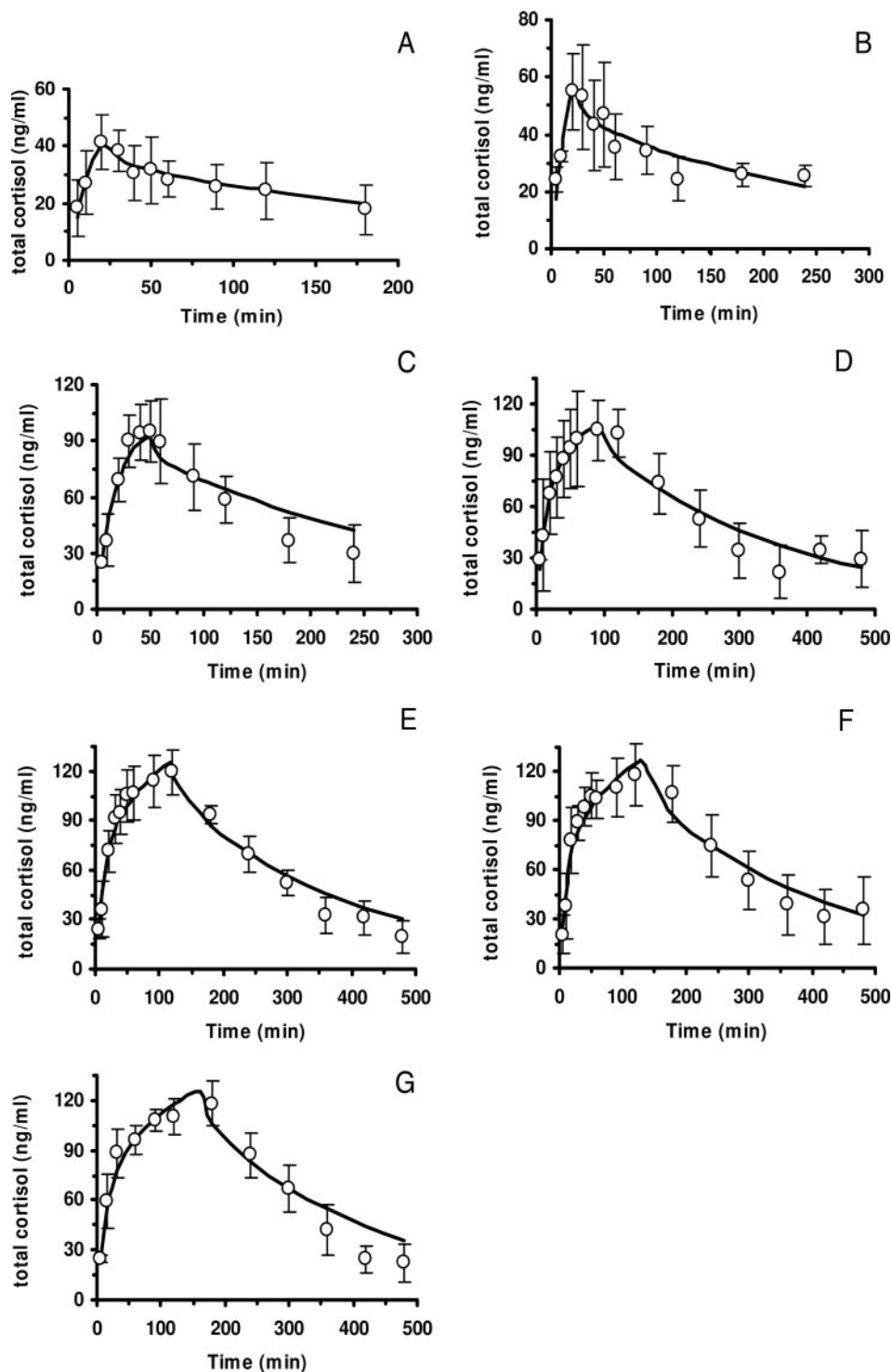


FIG. 3. Plasma concentration *vs.* time curves of total cortisol after iv administration of ACTH in six to eight horses. ACTH doses were 0.005 (A), 0.01 (B), 0.1 (C), 0.5 (D), 1 (E), 2 (F), and 10  $\mu\text{g}/\text{kg}$  (G). Experimental data (mean  $\pm$  SD) and fitted curves (—) are presented. Data were analyzed with a compartmental model taking into account the nonlinear disposition of cortisol and assuming that cortisol is released by an ACTH-stimulated adrenal gland according to an on/off zero order process over a given duration of time.

tegrate in a single set of equations the quantitative relationship existing between adrenal cortisol secretion and dose of iv ACTH and the *in vivo* nonlinear disposition of cortisol. The ultimate goal was to use this model to simulate the ARs to ACTH and explore various possible pathophysiological conditions known in man to modify the secretion and/or disposition of cortisol. This model offers a framework for interpretation of cortisol responses to rapid ACTH tests and

should provide valuable information of clinical relevance, especially to justify the choice of ACTH dose and select the most appropriate blood sampling time schedule for assessing adrenal gland responsiveness. For the present investigation, we used parameters actually obtained in horses by applying a nonradiolabeled method to estimate *in vivo* the adrenal gland secretion rate and corresponding cortisol disposition parameters (11). However, the same hypothesis regarding

**TABLE 2.** Parameters of CP by adrenals after iv ACTH in six to eight horses assuming a zero-order secretion process

ACTH dose ( $\mu\text{g}/\text{kg}$ )	RT (min)	$k_0$ [ $\mu\text{g}/(\text{kg}\cdot\text{min})$ ]	CP ( $\mu\text{g}/\text{kg}$ )
0.005	17	0.564	9.6
0.01	21	0.659	13.8
0.1	48	0.908	43.6
0.5	87	0.878	76.4
1	116	0.975	113
2	131	0.954	125
10	162	0.839	136

RT, Duration of cortisol secretion by adrenals. Values were obtained by fitting total cortisol plasma profiles after iv ACTH.

the validity or nonvalidity of free *vs.* total cortisol as a surrogate of adrenal gland response could be tested with our model but including sets of possible guest values obtained from the literature to extend our conclusions to other species, including man.

There is extensive literature describing the relationship between exogenous ACTH and cortisol concentrations, and various studies have provided an interpretation of the dose (concentration)-response curves in animals (23) or humans (12, 13, 24, 25). At the same time the importance of measuring free, not total, cortisol in ACTH stimulation tests is also gaining recognition (10, 26, 27). In our modeling approach, we combine a pharmacodynamic model and a pharmacokinetic model, both based on previous knowledge of cortisol secretion and disposition, that can reproduce and explain the AR to ACTH in terms of circulating free and total cortisol.

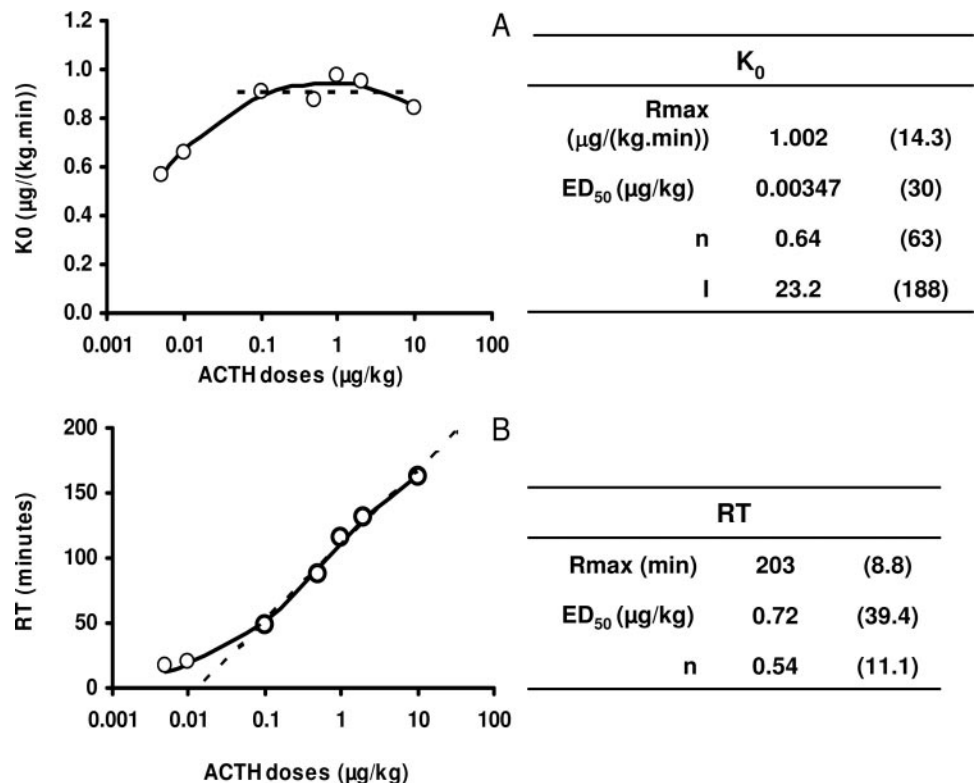
Several endpoints related to circulating cortisol concentrations have been used to describe the HPA axis response to various stimulations. These include snapshot cortisol con-

centrations, area under the total cortisol concentration *vs.* time curve, CP, peak cortisol concentration, or time to peak cortisol. Our model indicates that none of these endpoints alone is able to describe univocally the time course of the HPA axis response to ACTH stimulation. This is due to the conjunction of two unrelated properties of the investigated system, namely the nonlinear disposition of cortisol and the existence of two different mechanisms governing the adrenal gland response when stimulated by a low or high ACTH dose.

The nonlinearity of cortisol disposition is due to its saturable binding to CBG, the maximal binding capacity of CBG being exceeded during an ACTH test. This explains the lack of parallelism between the dose-effect curves expressed in terms of total *vs.* CBG-free cortisol. This is particularly evident when the selected endpoint is an AUC; in contrast, the distortion due to the nonlinearity is minimal when considering the peak cortisol concentration because, whatever the tested ACTH dose, the  $C_{\text{max}}$  is observed within a range of cortisol concentrations for which the CBG is saturated meaning that all maximal plasma cortisol concentrations were obtained in conditions where the cortisol disposition had become linear (see Fig. 2). The best approach to circumvent the confounding factor represented by the nonlinearity of total cortisol disposition is to take into account the nonlinear binding of cortisol to plasma CBG because more than 90% of the circulating cortisol in humans is bound to CBG (28), and the proportion of cortisol bound to CBG is from 67–87% in several domestic species including horses (17).

The part of the present model describing the nonlinear disposition of circulating cortisol due to the binding to CBG (see supplemental data) has been presented in detail else-

**FIG. 4.** Dose-response relationship between ACTH doses (0.005–10 mg/kg) and parameters of cortisol secretion by adrenals. A,  $k_0$ , zero order rate constant of cortisol secretion; B, RT, duration of cortisol secretion by adrenals. Experimental data (○) were obtained from the fitting of the post-ACTH cortisol profiles presented in Fig. 3. —, Fitted curves using equations 5 (A) and 6 (B). Inspection of A indicates that  $k_0$  reached a near-maximal value (---) over the (0.1–10  $\mu\text{g}/\text{kg}$ ) ACTH dose range, whereas RT (B) increased log-linearly (---) over the (0.1–10  $\mu\text{g}/\text{kg}$ ) ACTH dose range. Parameters of the dose-response relationship (equations 5 and 6) are presented:  $R_{\text{max}}$ , maximal response;  $\text{ED}_{50}$ , ACTH dose producing half of the maximal response;  $n$ , Hill coefficient;  $I$ , dimension-less parameter. Values shown are mean estimates (coefficients of variation, percentage).





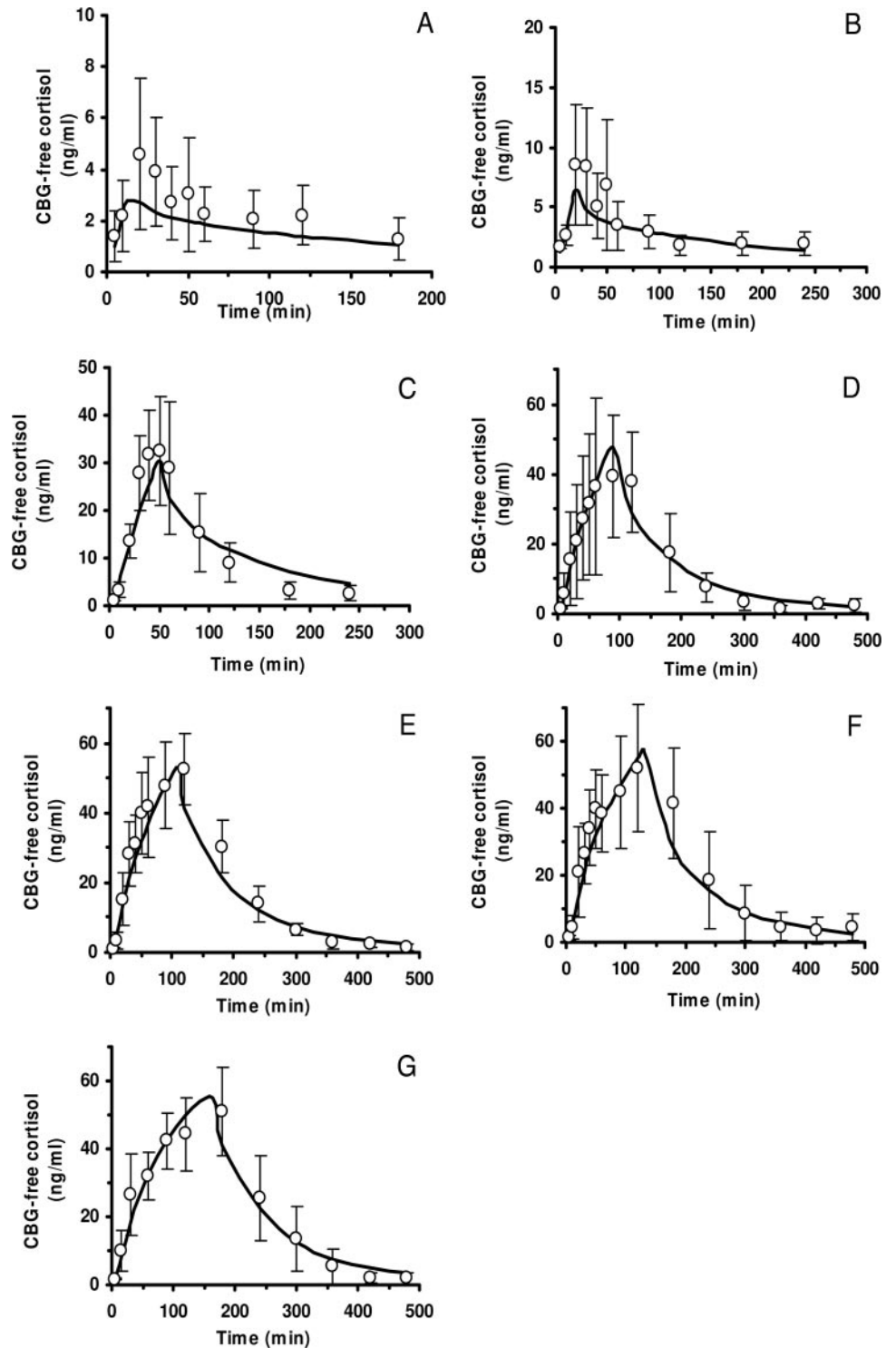


FIG. 5. Plasma concentration *vs.* time curves of CBG-free cortisol after iv administration of ACTH in six to eight horses. ACTH doses were 0.005 (A), 0.01 (B), 0.1 (C), 0.5 (D), 1 (E), 2 (F), and 10  $\mu\text{g}/\text{kg}$  (G). Experimental data (mean  $\pm$  SD) and simulated curves (—) are presented. Experimental data were obtained by calculation of  $\text{CBG}_{\text{free}}$  from measured total cortisol and estimated binding parameters. Simulated curves were obtained with the general pharmacokinetic/pharmacodynamic model taking into account both the nonlinear disposition of cortisol and the ACTH-dependent cortisol secretion by adrenals.

where (11). This model was used to fit the total plasma cortisol *vs.* time profiles observed after different doses of ACTH and enabled us to perform simulations to investigate the influence of alterations in cortisol binding to CBG on circulating cortisol profiles during rapid ACTH tests. We showed that modification of the CBG concentrations (*i.e.* the binding parameter,  $B_{\text{max}}$ ) induced changes in the simulated total cortisol concentration *vs.* time profiles (Fig. 8A). Con-

sidering their amplitude, such modifications seem highly relevant for the interpretation of rapid ACTH tests and confirm recent work suggesting that normal interindividual variations of CBG levels in healthy subjects can affect the results of rapid ACTH tests (10). As expected, the corresponding plasma concentration profiles of cortisol not bound to CBG showed little or no modification, rightly indicating the lack of alteration of the cortisol secretion process (Fig. 8C).

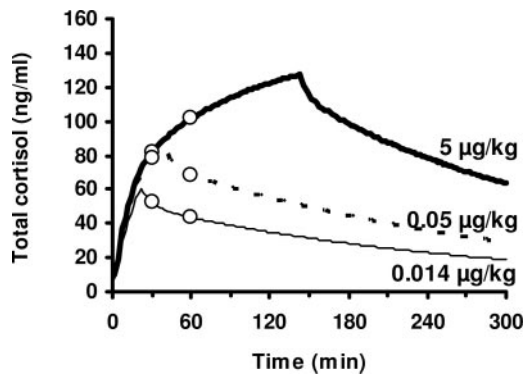


FIG. 6. Influence of the ACTH dose on the shape of the plasma concentration profiles of total cortisol. ACTH doses: 0.014 (*thin line*), 0.05 (*dashed line*), and 5 (*thick line*)  $\mu\text{g}/\text{kg}$ .  $\circ$ , Simulated total cortisol concentrations at 30 and 60 min post-ACTH. Inspection of the figure shows that the total plasma cortisol has the same value at 30 min post-ACTH after the two highest doses, whereas a later sampling (60 min) may be able to distinguish between these two dose levels.

An increasing number of studies report situations characterized by alterations of both HPA axis activity and plasma CBG levels. In particular, HPA axis activation and a decrease in CBG levels are observed during severe stress in patients with inflammation and burn injury (29), septic shock (9), or undergoing cardiac surgery (30). The same pattern has been described in other species, during acute stress in rats (31) or social stress in horses (32). Endocrinometabolic disorders such as obesity (33, 34) and insulin resistance syndrome (35) were also associated with both these alterations in HPA axis activity and plasma CBG levels. Our simulations to compare the physiological situation with one in which there was both an increase in adrenal cortisol secretion (increased  $k_0$ ) and a decreased CBG (decrease in  $B_{\text{max}}$ ) indicated that, when considering only the plasma concentration profiles of total cortisol, these changes tended to cancel each other, so that the HPA axis alteration was not detected by the ACTH test (Fig. 8B). Conversely, because the plasma concentration profiles of cortisol not bound to CBG were only controlled by the cortisol secretion process, the ACTH test was able to detect the HPA axis modification (Fig. 8D). Thus, as recently stated by Dhillo *et al.* (10), circulating cortisol levels should always be interpreted in reference to CBG concentrations, and the modeling approach proposed here should be very useful for investigating this point. An alternative is to select an analytical technique that specifically measures the plasma free cortisol concentration.

Whatever the analytical technique used for the cortisol measurement (free or total), the shape of the dose (concentration)-response curve linking the ACTH dose and the cortisol (free or total) response need to be understood to properly interpret the results of an ACTH test or/and select an optimal sampling time to discriminate between different adrenal gland conditions.

A major result of our modeling of adrenal gland secretion was to propose that cortisol secretion by the adrenals following ACTH stimulation is governed by both a dose-dependent  $k_0$  and a dose-dependent duration of adrenal gland secretion (RT).

These modeling results are consistent with the fact that the

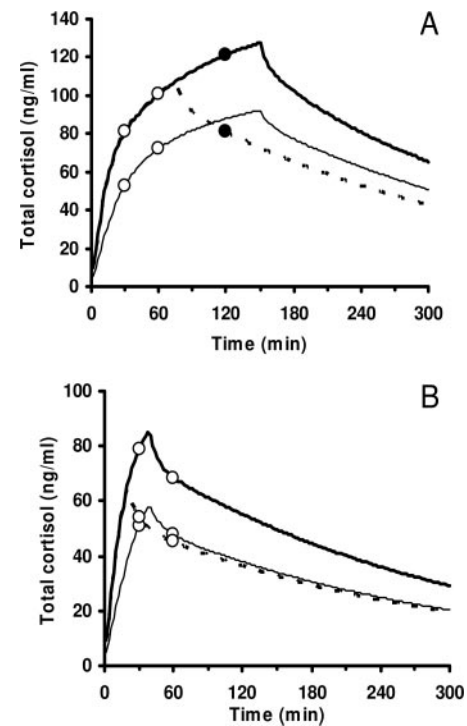
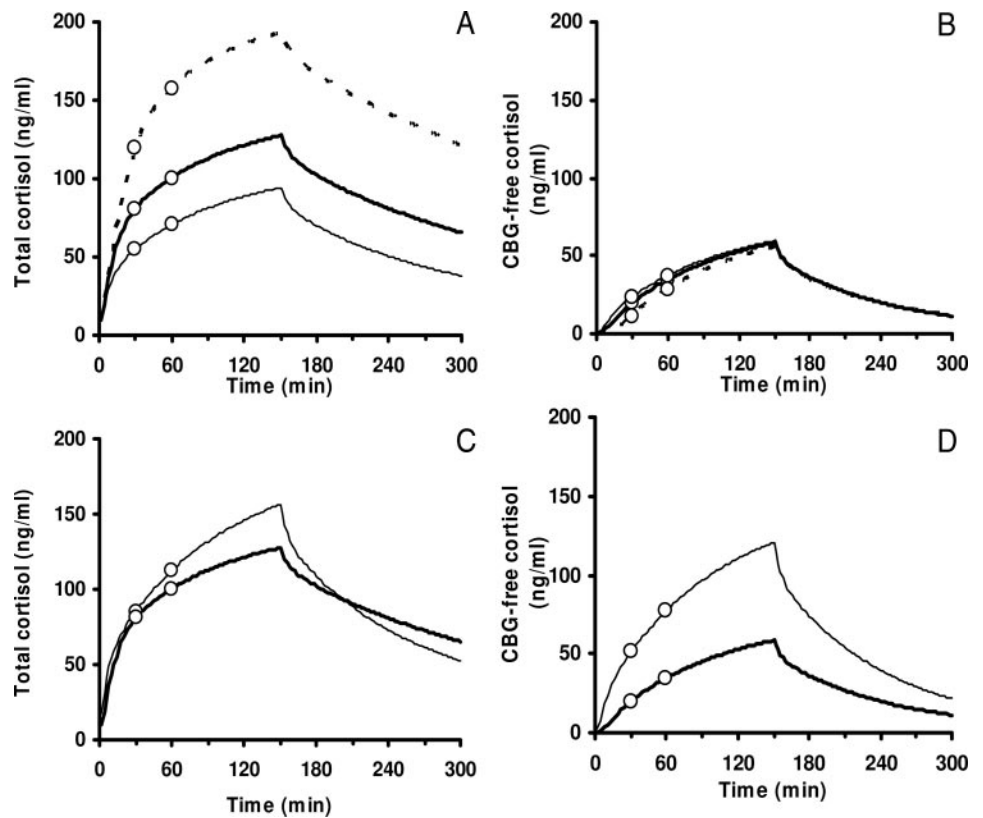


FIG. 7. Simulations of plasma concentration profiles of total cortisol after iv ACTH at 5 (A) and 0.05 (B)  $\mu\text{g}/\text{kg}$ . Three different conditions were simulated: plasma cortisol profile obtained with the cortisol secretion parameters ( $k_0$ , RT) estimated from experimental data (*thick line*), plasma cortisol profile obtained by halving  $k_0$  (*thin line*), and plasma cortisol profile obtained by halving RT (*dashed line*).  $\circ$ , Simulated total cortisol concentrations at 30 and 60 min post-ACTH;  $\bullet$ , simulated total cortisol concentrations at 120 min post-ACTH. Inspection of the figure indicates that an ACTH dose of 5  $\mu\text{g}/\text{kg}$  is appropriate to diagnose a condition characterized by a reduction of the secretion rate when samples are obtained at 30 and 60 min but not for a condition for which only the duration of the secretion rate is reduced except if a 120-min sample is collected. In contrast, for the small ACTH dose (B), the two pathological conditions are acknowledged with samples at 30 and 60 min, but their etiology (level or duration or secretion rate) remains indistinguishable.

adrenal glands do not accumulate a pool of releasable cortisol (for which a first order rate constant would be expected to describe the response to an ACTH administration) but instead stimulate a preestablished enzymatic pool, *i.e.* only increase cortisol synthesis (14). The zero order release of cortisol by the adrenals is also in agreement with previous studies indicating that plasma cortisol concentrations attained a plateau when high ACTH doses were administered as a bolus (13, 36, 37).

On the basis of the observed  $k_0$  variations in response to the range of tested ACTH doses (Table 2 and Fig. 4A), a constant value for  $k_0$  can be considered without loss of generality in the range of 0.1–10  $\mu\text{g}/\text{kg}$  ACTH. This indicates that the ACTH-induced increase in cortisol secretion rate rapidly attained a maximum and that beyond this, the increased CP in response to exogenous ACTH is only due to the increase in RT (Table 2). Consequently, when blood samples are taken before the end of adrenal secretion, our model predicts that the measured cortisol concentrations will be the same whatever the administered ACTH dose within this range. In particular, the simulated plasma cortisol concen-

FIG. 8. Simulations of plasma concentration profiles of total (A and C) and CBG-free (B and D) cortisol after an iv dose of ACTH at 5  $\mu\text{g}/\text{kg}$ . A and B, Plasma cortisol profiles obtained with the cortisol secretion parameters ( $k_0$ , RT) and the CBG-binding capacity ( $B_{\text{max}}$ ) estimated from experimental data (*thick line*), plasma cortisol profiles obtained by halving (*thin line*) or doubling (*dashed line*)  $B_{\text{max}}$ . C and D, Plasma cortisol profiles obtained with the cortisol secretion parameters ( $k_0$ , RT) and the CBG-binding capacity ( $B_{\text{max}}$ ) estimated from experimental data (*thick line*), plasma cortisol profiles obtained by simultaneously halving  $B_{\text{max}}$  and doubling  $k_0$  (*thin line*).  $\circ$ , Simulated total cortisol concentrations at 30 and 60 min post-ACTH. Visual inspection of A and B indicates that CBG concentration has a major impact on the total plasma cortisol concentration but no effect on CBG-free cortisol. C and D, Conditions characterized by both an increase of cortisol secretion rate and a decrease of CBG concentration are not distinguishable from the measurement of the total plasma cortisol concentration but easily detected from the CBG-free plasma cortisol.



trations at 30 min after ACTH were identical for the 5 and 0.05  $\mu\text{g}/\text{kg}$  ACTH doses, which are equivalent to the high and low doses used for ACTH stimulation tests in humans (Fig. 6). These findings are in full agreement with reports indicating that cortisol concentrations 30 (or 20) min after ACTH did not differ after standard low (0.5  $\mu\text{g}/\text{m}^2$ )- and high (250  $\mu\text{g}$ )-dose ACTH tests in healthy humans (3, 4, 6, 13). In the same way, the ability of the low-dose ACTH test in humans to stimulate maximal adrenal secretion up to 30 min postinjection (13, 38) can be interpreted in the framework of our model as the capacity of a low dose of ACTH to induce maximal  $k_0$ , a higher ACTH dose only being able to increase RT (and thereby the overall CP).

Moreover, it has been described in normal subjects that cortisol levels at 60 min were lower than those at 30 min after a low dose of ACTH, whereas the inverse was observed after the high-dose ACTH test (4, 6, 38, 39). These observations can be reproduced in our model by simply assuming an identical  $k_0$ , and an RT of 30–60 min for the low ACTH dose and more than 60 min for the high ACTH dose (Fig. 6; 0.05  $\mu\text{g}/\text{kg}$  for the low ACTH doses and 5  $\mu\text{g}/\text{kg}$  for the high ACTH doses).

Another matter of debate concerning standard ACTH tests is their ability to discriminate between patients with a normal or insufficient HPA axis. Several reports have concluded that both low- and high-dose ACTH tests failed to effectively detect mild degrees of secondary adrenal insufficiency (6–8). The simulations performed here lead to similar conclusions if adrenal insufficiency corresponds to an unchanged  $k_0$  associated solely with a decrease in RT, so that the cortisol levels at 30 or 60 min are not modified (Fig. 7A). The framework permitting interpretation of some adrenal insufficien-

cies in terms of decreased RT is consistent with clinical reports of patients showing an insufficient overall cortisol response to sustained stress but a normal response to rapid ACTH tests (5, 7, 40).

The main factor able to control the duration of maximal secretion is the kinetic disposition of ACTH and the fact that RT increased log-linearly with the ACTH dose (Fig. 4) would be expected if the duration of the adrenal gland secretion is an on/off process relying on some critical threshold plasma ACTH concentration. One consequence of this is that the selection of sampling time is of particular importance for the screening performance of both low- and high-dose ACTH tests. The choice of the sampling times used in standard ACTH tests has been justified by the correlations between cortisol concentrations at 30 min after high ACTH doses and peak cortisol concentrations during insulin hypoglycemia (1, 41), but this continues to be debated (2, 6). Our simulations strongly suggest that information about the shape of the cortisol concentration *vs.* time profiles in response to ACTH, and particularly about the physiological value of RT, are essential to select appropriate sampling times for the detection of adrenal insufficiencies characterized by blunted capacities to maintain prolonged cortisol secretion. Moreover, direct investigation of the ACTH kinetic disposition could be helpful in discriminating between an altered ACTH disposition and a decrease in adrenal gland responsiveness, leading to an alteration of the threshold ACTH concentration at which cortisol secretion is initiated.

In conclusion, this study demonstrated the value of a pharmacokinetic/pharmacodynamic model describing the relation between iv ACTH and AR reflected by plasma cortisol

concentration *vs.* time profiles. Although further experiments will be needed to investigate this modeling approach in humans, our simulations of the conditions encountered in evaluating the human HPA axis suggest that such an approach should increase the understanding of AR to ACTH tests and thus help to improve their clinical relevance.

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### References

- Lindholm J, Kehlet H, Blichert-Toft M, Dinesen B, Riishede J 1978 Reliability of the 30-minute ACTH test in assessing hypothalamic-pituitary-adrenal function. *J Clin Endocrinol Metab* 47:272–274
- Grinspoon SK, Biller BM 1994 Clinical review 62: laboratory assessment of adrenal insufficiency. *J Clin Endocrinol Metab* 79:923–931
- Broide J, Soferman R, Kivity S, Golander A, Dickstein G, Spirer Z, Weisman Y 1995 Low-dose adrenocorticotropin test reveals impaired adrenal function in patients taking inhaled corticosteroids. *J Clin Endocrinol Metab* 80:1243–1246
- Tordjman K, Jaffe A, Grazas N, Apter C, Stern N 1995 The role of the low dose (1 microgram) adrenocorticotropin test in the evaluation of patients with pituitary diseases. *J Clin Endocrinol Metab* 80:1301–1305
- Streeten DH, Anderson Jr GH, Bonaventura MM 1996 The potential for serious consequences from misinterpreting normal responses to the rapid adrenocorticotropin test. *J Clin Endocrinol Metab* 81:285–290
- Mayenknecht J, Diederich S, Bahr V, Plockinger U, Oelkers W 1998 Comparison of low and high dose corticotropin stimulation tests in patients with pituitary disease. *J Clin Endocrinol Metab* 83:1558–1562
- Streeten DH 1999 Shortcomings in the low-dose (1 µg) ACTH test for the diagnosis of ACTH deficiency states. *J Clin Endocrinol Metab* 84:835–837
- Suliman AM, Smith TP, Labib M, Fiad TM, McKenna TJ 2002 The low-dose ACTH test does not provide a useful assessment of the hypothalamic-pituitary-adrenal axis in secondary adrenal insufficiency. *Clin Endocrinol (Oxf)* 56:533–539
- Beishuizen A, Thijs LG, Vermes I 2001 Patterns of corticosteroid-binding globulin and the free cortisol index during septic shock and multitrauma. *Intensive Care Med* 27:1584–1591
- Dhillon WS, Kong WM, Le Roux CW, Alagband-Zadeh J, Jones J, Carter G, Mendoza N, Meeran K, O'Shea D 2002 Cortisol-binding globulin is important in the interpretation of dynamic tests of the hypothalamic-pituitary-adrenal axis. *Eur J Endocrinol* 146:231–235
- Picard-Hagen N, Gayraud V, Alvinerie M, Smeyers H, Ricou R, Bousquet-Mélou A, Toutain PL 2001 A nonlabeled method to evaluate cortisol production rate by modeling plasma CBG-free cortisol disposition. *Am J Physiol* 281:E946–E956
- Oelkers W, Boelke T, Bahr V 1988 Dose-response relationships between plasma adrenocorticotropin (ACTH), cortisol, aldosterone, and 18-hydroxycorticosterone after injection of ACTH-(1–39) or human corticotropin-releasing hormone in man. *J Clin Endocrinol Metab* 66:181–186
- Arvat E, Di Vito L, Lanfranco F, Maccario M, Baffoni C, Rossetto R, Aimaretti G, Camanni F, Ghigo E 2000 Stimulatory effect of adrenocorticotropin on cortisol, aldosterone, and dehydroepiandrosterone secretion in normal humans: dose-response study. *J Clin Endocrinol Metab* 85:3141–3146
- Orth DN, Kovacs J 1998 The adrenal cortex. In: Wilson JD, Foster DW, Kronenberg HM, Reed Larsen P, eds. *Williams textbook of endocrinology*. 9th ed. Philadelphia: W.B. Saunders Co.; 517–664
- Catalano RD, Stuve L, Ramachandran J 1986 Characterization of corticotropin receptors in human adrenocortical cells. *J Clin Endocrinol Metab* 62:300–304
- Kapas S, Cammas FM, Hinson JP, Clark AJ 1996 Agonist and receptor binding properties of adrenocorticotropin peptides using the cloned mouse adrenocorticotropin receptor expressed in a stably transfected HeLa cell line. *Endocrinology* 137:3291–3294
- Gayraud V, Alvinerie M, Toutain PL 1996 Interspecies variations of corticosteroid-binding globulin parameters. *Domest Anim Endocrinol* 13:35–45
- Lassourd V, Gayraud V, Laroute V, Alvinerie M, Benard P, Courtot D, Toutain PL 1996 Cortisol disposition and production rate in horses during rest and exercise. *Am J Physiol* 271:R25–R33
- Toutain PL, Lefebvre HP, King JN 2000 Benazepril disposition and effect in dogs revisited with a pharmacokinetic/pharmacodynamic modeling approach. *J Pharmacol Exp Ther* 292:1087–1093
- Tait JF, Burnstein S 1964 In vivo studies of steroids dynamics in man. In: Pincus G, Thiman KV, Astwood EB, eds. *The hormones*. New York: Academic Press; 441–557
- Keller F, Giehl M, Czock D, Zellner D 2002 PK-PD curve-fitting problems with the Hill equation? Try one of the 1-exp functions derived from Hodgkin, Douglas or Gompertz. *Int J Clin Pharmacol Ther* 40:23–29
- Cornish-Bowden A 1995 *Fundamentals of enzyme kinetics*. London: Portland Press Ltd.
- Verkerk GA, Macmillan KL, McLeay LM 1994 Adrenal cortex response to adrenocorticotropin hormone in dairy cattle. *Domest Anim Endocrinol* 11:115–123
- Oelkers W 1996 Dose-response aspects in the clinical assessment of the hypothalamic-pituitary-adrenal axis, and the low-dose adrenocorticotropin test. *Eur J Endocrinol* 135:27–33
- Pasquali R, Ambrosi B, Armanini D, Cavagnini F, Uberti ED, Del Rio G, de Pergola G, Maccario M, Mantero F, Marugo M, Rotella CM, Vettor R 2002 Cortisol and ACTH response to oral dexamethasone in obesity and effects of sex, body fat distribution, and dexamethasone concentrations: a dose-response study. *J Clin Endocrinol Metab* 87:166–175
- Hamrahian AH, Oseni TS, Arafah BM 2004 Measurements of serum free cortisol in critically ill patients. *N Engl J Med* 350:1629–1638
- le Roux CW, Chapman GA, Kong WM, Dhillon WS, Jones J, Alagband-Zadeh J 2003 Free cortisol index is better than serum total cortisol in determining hypothalamic-pituitary-adrenal status in patients undergoing surgery. *J Clin Endocrinol Metab* 88:2045–2048
- Hammond GL, Smith CL, Paterson NA, Sibbald WJ 1990 A role for corticosteroid-binding globulin in delivery of cortisol to activated neutrophils. *J Clin Endocrinol Metab* 71:34–39
- Garrel DR 1996 Corticosteroid-binding globulin during inflammation and burn injury: nutritional modulation and clinical implications. *Horm Res* 45:245–251
- Roth-Isigkeit AK, Dibbelt L, Schmucker P 2000 Blood levels of corticosteroid-binding globulin, total cortisol and unbound cortisol in patients undergoing coronary artery bypass grafting surgery with cardiopulmonary bypass. *Steroids* 65:513–520
- Fleshner M, Deak T, Spencer RL, Laudenslager ML, Watkins LR, Maier SF 1995 A long-term increase in basal levels of corticosterone and a decrease in corticosteroid-binding globulin after acute stressor exposure. *Endocrinology* 136:5336–5342
- Alexander SL, Irvine CH 1998 The effect of social stress on adrenal axis activity in horses: the importance of monitoring corticosteroid-binding globulin capacity. *J Endocrinol* 157:425–432
- Vicennati V, Pasquali R 2000 Abnormalities of the hypothalamic-pituitary-adrenal axis in nondepressed women with abdominal obesity and relations with insulin resistance: evidence for a central and a peripheral alteration. *J Clin Endocrinol Metab* 85:4093–4098
- Bjorntorp P 1999 Neuroendocrine perturbations as a cause of insulin resistance. *Diabetes Metab Res Rev* 15:427–441
- Fernandez-Real JM, Grasa M, Casamitjana R, Ricart W 2000 The insulin resistance syndrome and the binding capacity of cortisol binding globulin (CBG) in men and women. *Clin Endocrinol (Oxf)* 52:93–99
- Daïdoh H, Morita H, Mune T, Murayama M, Hanafusa J, Ni H, Shibata H, Yasuda K 1995 Responses of plasma adrenocortical steroids to low dose ACTH in normal subjects. *Clin Endocrinol (Oxf)* 43:311–315
- Keller-Wood ME, Shinsako J, Dallman MF 1983 Integral as well as proportional adrenal responses to ACTH. *Am J Physiol* 245:R53–R59
- Nye EJ, Grice JE, Hockings GI, Strakosch CR, Crosbie GV, Walters MM, Jackson RV 1999 Comparison of adrenocorticotropin (ACTH) stimulation tests and insulin hypoglycemia in normal humans: low dose, standard high dose, and 8-hour ACTH-(1–24) infusion tests. *J Clin Endocrinol Metab* 84:3648–3655
- Kelestimur F, Goktas Z, Gulmez I, Unluhizari K, Bayram F, Ozesmi M, Guven M, Tutus A 2000 Low dose (1 microg) adrenocorticotropin stimulation test in the evaluation of hypothalamic-pituitary-adrenal axis in patients with active pulmonary tuberculosis. *J Endocrinol Invest* 23:235–239
- Streeten DH, Anderson Jr GH, Dalakos TG, Seeley D, Malloy JS, Eusebio R, Sunderlin FS, Badawy SZ, King RB 1984 Normal and abnormal function of the hypothalamic-pituitary-adrenocortical system in man. *Endocr Rev* 5:371–394
- Lindholm J, Kehlet H 1987 Re-evaluation of the clinical value of the 30 min ACTH test in assessing the hypothalamic-pituitary-adrenocortical function. *Clin Endocrinol (Oxf)* 26:53–59

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