The principle of homeostasis in the hypothalamus-pituitary-adrenal system: new insight from positive feedback


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Peters A, Conrad M, Hubold C, Schweiger U, Fischer B, Fehm HL. The principle of homeostasis in the hypothalamus-pituitary-adrenal system: new insight from positive feedback. Am J Physiol Regul Integr Comp Physiol 293: R83–R98, 2007. First published April 25, 2007; doi:10.1152/ajpregu.00907.2006.—Feedback control, both negative and positive, is a fundamental feature of biological systems. Some of these systems strive to achieve a state of equilibrium or “homeostasis”. The major endocrine systems are regulated by negative feedback, a process believed to maintain hormonal levels within a relatively narrow range. Positive feedback is often thought to have a destabilizing effect. Here, we present a “principle of homeostasis,” which makes use of both positive and negative feedback loops. To test the hypothesis that this homeostatic concept is valid for the regulation of cortisol, we assessed experimental data in humans with different conditions (gender, obesity, endocrine disorders, medication) and analyzed these data by a novel computational approach. We showed that all obtained data sets were in agreement with the presented concept of homeostasis in the hypothalamus-pituitary-adrenal axis. According to this concept, a homeostatic system can stabilize itself with the help of a positive feedback loop. The brain mineralocorticoid and glucocorticoid receptors—with their known characteristics—fulfill the key functions in the homeostatic concept: binding cortisol with high and low affinities, acting in opposing manners, and mediating feedback effects on cortisol. This study supports the interaction between positive and negative feedback loops in the hypothalamus-pituitary-adrenal system and in this way sheds new light on the function of dual receptor regulation. Current knowledge suggests that this principle of homeostasis could also apply to other biological systems.

mathematical modeling; mineralocorticoid receptor; glucocorticoid receptor; obesity

Hormones, neuropeptides, neurotransmitters, and growth factors help the organism to achieve a state of equilibrium, which is referred to as homeostasis (18). These messengers (e.g., glucocorticoids, neurotrophins) typically bind as ligands to at least two different kinds of receptors that display different affinities and in this way convey their stabilizing signals. The principle, however, that underlies such homeostatic processes and that may explain why there are two receptors, is a longstanding mystery. Therefore, we assessed experimental data and analyzed these by a novel computational approach, showing that the data were in agreement with a fundamental homeostatic principle in the hypothalamic-pituitary-adrenal (HPA) system. In addition, we provide evidence from the literature that this concept could apply to other biological systems.

Usually, equilibria are thought to be driven by negative feedback only, for example, a deviated pendulum suffers a retrieving force to its stationary state. Biological systems, however, often display the typical components: a ligand, a low-affinity receptor, and a high-affinity receptor. The presence of such components suggests a general “principle of homeostasis” which includes positive feedback in particular ranges of ligand concentrations. As a recent discovery, positive feedback has been shown to be a ubiquitous signal transduction motif that allows systems to convert graded inputs into decisive, all-or-none outputs (2, 13). Thus, focusing on positive feedback already has shed light on another class of processes, that is, nonhomeostatic “multistable” processes like cellular on-off switches. Here we elaborate a basic model of homeostasis and introduce positive feedback loops as key mechanisms of homeostasis (Fig. 1, A and B; see also Box 1).

Which components of the HPA system fulfill the functions of A, B, and C in the general rules (Box 1)? The brain-pituitary system via ACTH stimulates the adrenal glands, increasing cortisol (Fig. 1C). In a feedback loop, cortisol acts on the brain by binding as a ligand to cerebral glucocorticoid receptors: at low concentrations, cortisol binds to the mineralocorticoid receptor (MR), and at higher concentrations, cortisol binds to the glucocorticoid receptor (GR) (Fig. 1D). MR and GR descend from a gene duplication deep in the vertebrate lineage and function as dual cortisol sensors (14). Starting in the year 1968 with the milestone paper of Bruce McEwen and colleagues (67), a large number of researchers have since managed to characterize the two brain receptor subtypes both biochemically and functionally. High densities of MR were found in the hippocampus, dentate gyrus, lateral septum, and amygdala, while abundant GR were detected in all brain regions (74). It is known that the two corticosteroid receptors MR and GR operate in an opposing manner on several processes such as synaptic transmission (28), synaptic plasticity (4, 52, 73, 77, 92), and cell survival (25). It is unknown, however, whether brain MR and GR also operate counteractively in the central feedback on the HPA system. Some scientists have referred to pharmacological studies (3, 31, 35, 47, 73, 81, 89, 99) and have concluded that MR and GR both exert inhibitory feedback in the HPA system. Ron de Kloet and colleagues (28) emphasized the “proactive” mode of MR and the “reactive” mode of GR and that the balance between MR and GR is essential for cell homeostasis, mental performance, and health.

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Nevertheless, he did not bring up positive feedback in this context. Considering the opposing nature of MR and GR in other biological processes, we assume here that brain MR and GR make use of positive and negative feedback in the HPA system, respectively. We, therefore, formulate the homeostatic rules with respect to the HPA system by replacing A, B, and C by cortisol, MR, and GR (Box 2).

To test the hypothesis that the “principle of homeostasis” applies to the regulation of cortisol, we applied a computational approach to experimental data (Fig. 2):
Box 1

THE PRINCIPLE OF BIOLOGICAL HOMEOSTASIS

A, B, and C are molecules: one ligand, two receptors.

**Rule 1:** The ligand A binds with high affinity to a receptor B, but
with low affinity to a receptor C.

**Rule 2:** The two ligand-bound receptors act in opposing manners.

**Rule 3:** Ligand-bound B receptors increase\(^*\) while ligand-bound
C receptors decrease\(^*\) the concentration of A.

These interactions result in a homeostatic state of A.

\(^*\)Positive feedback; \(^\dagger\)negative feedback

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**METHODS**

**Differential equations.** To mathematically analyze experimental
data sets, we established a system involving two differential
equations. The rationale for the choice of terms in the equations is based
on the following aspects: Basically, the equations represent the three
rules (Box 2). We used a limited number of extensions that take into
account specific physiological features of the HPA system, that is,
ACTH as an intermediary signal posited in the brain-to-adrenal pathway,
a clearance of hormones from blood, and a peripheral feedback at
the adrenal level. As commented in the discussion, we deliberately
abstained from adding extra compartments (regions of the brain) or
extraregulatory processes (e.g., heterodimerization). For the denotations
used in the equations, see Table 1:

\[
\frac{d\text{ACTH}(t)}{dt} = c_1 \cdot \text{ACTH}(t) + \frac{M^\text{brain}_{\text{max}} \cdot \text{CORT}(t - \tau_1)}{\text{CORT}(t - \tau_1) + r \cdot M^\text{esc}_{\text{max}}} + r \cdot \text{MR}_{\text{esc}} + GR^\text{brain}_{\text{max}} \cdot \text{GR}_{\text{esc}} + r \cdot e(t) (1)
\]

The term \([d\text{ACTH}(t)/dt]\) denotes the derivative of plasma ACTH
concentrations over time \(t\). This change of ACTH in time depends on
1) ACTH and its first-order clearance \(c_1\) from plasma (see Table 1),
2) a positive MR-mediated effect, which is a function of \(\text{CORT}(t - \tau_1)\) (\(\tau_1\) indicates a delay time) and the pharmacokinetic parameters
\(M^\text{brain}_{\text{max}}\), \(M^\text{esc}_{\text{max}}\), \(r\), \(\text{MR}_{\text{esc}}\), and \(\text{GR}_{\text{esc}}\).

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**Box 2**

THE PRINCIPLE OF HOMEOSTASIS IN THE HPA SYSTEM

**Rule 1:** Cortisol binds at low concentrations to the brain MR and
only at high concentrations to the brain GR.

**Rule 2:** Activated brain MR and GR operate in an opposing
manner.

**Rule 3:** Cortisol raises\(^*\) its own serum concentration via ac-

tivated brain MR, while it reduces\(^*\) it via activated brain GR.

*Positive feedback; \(^\dagger\)negative feedback
The participants were instructed to abstain from alcohol, not to perform any kind of exhausting physical activity, and to go to bed no later than 11:00 PM on the day preceding the study. On the days of the experiments, subjects arrived at noon at the medical research unit and received a standardized meal. A cannula was inserted into an antecubital vein at 12:00 PM. Afterward, each participant remained fasted except for the free intake of water. Three baseline blood samples for determining plasma ACTH and serum cortisol were collected from 3:30 PM until 4:00 PM. After the baseline period, CRH (CRH Ferring, Ferring Arzneimittel, Kiel, Germany) was intravenously injected at 4:00 PM at a dose of 1 μg/kg adapted to body mass. Blood samples were subsequently drawn at short intervals of 5 min until 5:30 PM and at intervals of 15 min until the end of the test at 8:00 PM.

All blood samples were immediately centrifuged and the supernatants were stored at −24°C until assaying. Cortisol serum concentrations were determined by electrochemiluminescence immunoassay in conjunction with the Elecsys 2010 (Roche Molecular Biochemical Diagnostics, Mannheim, Germany; intraassay CV 1.3%; interassay CV 3.8%). Corticosteroid-binding globulin (CBG) was measured by a highly sensitive RIA (IBL Immuno-Biological Laboratories, Hamburg, Germany; intraassay CV < 3.8% and interassay CV < 5.0%).

The experimental methods that we had used in the study on MR blockade and the study on patients with patients with Addison’s and Cushing’s disease are described in the supplementary methods, which can be found online at the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology website.

Parameter identification. The parameter identification method is a mathematical procedure for obtaining optimal parameters from the comparison of experimental data and a system of differential equations. The parameter identification procedure repeats three steps until an optimal parameter set has been found. We begin with a set of n parameters on an n-dimensional grid.

In the first step of the algorithm, approximate concentration profiles ACTH(t) and CORT(t) are created. For this purpose, conventional numeric “delay differential-equation-solvers” are employed (9, 46). In a second step, the algorithm compares the differences between the obtained concentration profiles with the actual experimental ACTH and cortisol data from a test subject. This difference provides a numeric “delay differential-equation-solver” with the help of data-based parameter identification and the differential equations. B: CRH challenge in obese and healthy men. C: treatment with canrenoate or placebo in healthy men. Inset, right: feedback-analysis illustrates that the biphasic dose-response curve (cortisol → change in ACTH in time) is shifted to the right. With the right shift of the biphasic curve, the brain setpoint of the HPA system (vertical arrow) is altered, and homeostasis is achieved only at higher cortisol concentrations. D: continuous hydrocortisone infusion (50 mg/h, 0–120 min) in patients with Addison’s disease or in patients with Cushing’s disease/ADX. Inset, right: note the broad range of cortisol concentrations that is covered by experimental observations (shaded area). Error bars indicate SE.

Fig. 3. Experimental data and theoretical predictions of ACTH and cortisol concentrations during various challenge tests. In all conditions, the experimental data (data dots), computed the prediction error, and tested the hypothesis (significant prediction error).
measure for the prediction error. The difference is calculated using the root-mean-square procedure. The third step of the algorithm modifies the parameter values so that the prediction error decreases. Here, we employed what we refer to as direct exploratory methods of “numeric optimization” (39).

These three steps are repeated until the difference can no longer be reduced. Then we carry out this procedure for all points of the $n$-dimensional grid. The outcome of the parameter identification method is the parameter set that provides the lowest prediction error. A prediction error $<0.05$ is considered as high agreement between experimental data and theoretical prediction. In the sense of the used error measure, an error $<0.20$ can be regarded as satisfactory, a larger error as unacceptable agreement. We apply this procedure for every test individual separately. The parameter identification was done by MATLAB 7.0.

Statistical analysis. To compare data among different groups of subjects, we used the following statistical tools: Dependent and independent Student’s $t$-tests were used for comparing characteristics and identified parameters between subject groups. One sample $t$-test was employed to test whether the mean of one variable differs from a constant. ANOVA was performed for comparing parameters among three groups. $P$ values $<0.05$ were considered to be significant. Values are expressed as means $\pm$ SE. The analysis was done with SPSS 12.0.

RESULTS

Experiments in healthy humans. When CRH was administered to healthy subjects (characteristics found online in supplementary Table 1), ACTH profiles in men and women were
similar, although women had slightly higher cortisol concentrations in the recovery phase (Fig. 3A, left). The ACTH curves reflect the combined effects of CRH and cortisol on the brain-pituitary system. The cortisol profiles reflect the combined effects of ACTH and cortisol on the adrenal gland. In women, cortisol concentrations were higher than in men, suggesting that the brain corticosteroid feedback was different between the sexes. When the parameter identification procedure was applied to these data, the parameter $MP_{\text{brain}}^{\text{max}}$ was found to be higher $(2.3 \pm 0.1$ vs. $2.0 \pm 0.1$ fmol/l $^{-1}$ s$^{-1}$; $P < 0.05$) and $GR_{\text{E}}^{\text{max}}$ lower $(2.8 \pm 0.1$ vs. $3.2 \pm 0.1$ fmol/l $^{-1}$ s$^{-1}$; $P < 0.05$) in healthy women than in healthy men (Table 2). To interpret these results easier, we calculated the ratio between $MP_{\text{brain}}^{\text{max}}$ and $GR_{\text{E}}^{\text{max}}$ as an estimate of the MR:GR balance; the balance was larger in healthy women $(1:1.22)$ compared with healthy men $(1:1.60)$. Note that $MR_{\text{E}}^{\text{max}}$ quantifies a positive and $GR_{\text{E}}^{\text{max}}$ a negative feedback (see Eq. 1).

When the identified parameters were used to reconstruct the ACTH and cortisol profiles, these theoretic predictions were similar to the observed data (prediction error < 0.05; Fig. 3A, right; Table 2). When analyzing how the variability of estimated parameters affected the predictions, we found that changes in the central parameters $MP_{\text{brain}}^{\text{max}}, GR_{\text{E}}^{\text{max}}$, and $CRH_{\text{E}}^{\text{exog}}$ sensitively influenced the predicted ACTH and cortisol concentrations (Fig. 4, A and B), while changes in the peripheral parameters $ACTH_{\text{E}}^{\text{adrenal}}$, and $GR_{\text{E}}^{\text{adrenal}}$ mainly affected predicted serum cortisol (Fig. 4, C–E). Systematic variation of $MR_{\text{E}}^{\text{max}}$ and $GR_{\text{E}}^{\text{max}}$ revealed a high robustness of the prediction since there is only one parameter set representing the best model fit (Fig. 5).

When the identified parameters were used to analyze the dose-response function for the brain corticosteroid feedback, women had a weaker inhibitory GR effect than men (inset in Fig. 3A). In both sexes, the maximum MR-mediated positive feedback effects were evident at a low serum cortisol concentration of 50 nmol/l (typical value at bedtime), whereas at 300 nmol/l (typical value at wake-up time) the counteracting MR- and GR effects were of equal strength, and at 600 nmol/l (typical value during a stressful event), the negative feedback effect of GR predominated. The theoretically determined weaker central feedback inhibition in women reflects the observation that they had higher cortisol compared with men. In this experiment, we found that a model of the HPA system that makes use of posi-

### Table 1. Functions, parameters, and constants used in the differential equations

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$ACTH(t)$</td>
<td>Concentration of plasma ACTH</td>
<td>Variable (dependent on time)</td>
<td>pmol</td>
<td></td>
</tr>
<tr>
<td>$CORT(t)$</td>
<td>Concentration of serum cortisol</td>
<td>Variable (dependent on time)</td>
<td>nmol</td>
<td></td>
</tr>
<tr>
<td>$MR_{\text{brain}}^{\text{max}}$</td>
<td>Maximal efficacy of cortisol-bound brain/pituitary MR on ACTH</td>
<td>To be determined individually in respective subject</td>
<td>fmol$^{-1}$ s$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$GR_{\text{brain}}^{\text{max}}$</td>
<td>Maximal efficacy of cortisol-bound brain/pituitary GR on ACTH</td>
<td>To be determined individually in respective subject</td>
<td>fmol$^{-1}$ s$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$CRH_{\text{E}}^{\text{exog}}$</td>
<td>Efficacy of exogenous CRH on ACTH</td>
<td>To be determined individually in respective subject</td>
<td>pmol$^{-1}$ s$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$ACTH_{\text{pituitary}}^{\text{max}}$</td>
<td>Efficacy of pituitary ACTH on cortisol</td>
<td>To be determined individually in respective subject</td>
<td>pmol$^{-1}$ s$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$GR_{\text{pituitary}}^{\text{max}}$</td>
<td>Efficacy of cortisol-bound adrenal GR on cortisol</td>
<td>To be determined individually in respective subject</td>
<td>pmol$^{-1}$ s$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$c_1$</td>
<td>Clearance rate of plasma ACTH</td>
<td>$\log(2)$</td>
<td>s$^{-1}$</td>
<td>(45, 91)</td>
</tr>
<tr>
<td>$c_2$</td>
<td>Clearance rate of serum cortisol</td>
<td>$\log(2)$</td>
<td>s$^{-1}$</td>
<td>(30)</td>
</tr>
<tr>
<td>$\tau_1$</td>
<td>Delay time in the feedback pathway</td>
<td>60 - 20</td>
<td>120</td>
<td>s</td>
</tr>
<tr>
<td>$\tau_2$</td>
<td>Delay time in the forward pathway</td>
<td>72</td>
<td>s</td>
<td>(82)</td>
</tr>
<tr>
<td>$MR_{\text{pituitary}}^{\text{max}}$</td>
<td>Concentration of free cortisol that evokes half-maximal MR response on ACTH</td>
<td>0.5</td>
<td>nmol/l</td>
<td></td>
</tr>
<tr>
<td>$GR_{\text{pituitary}}^{\text{max}}$</td>
<td>Concentration of free cortisol that evokes half-maximal GR response on ACTH</td>
<td>5.0</td>
<td>nmol/l</td>
<td>(82)</td>
</tr>
<tr>
<td>$q$</td>
<td>Conversion factor for adrenal inputs</td>
<td>1</td>
<td>pmol/l</td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>Ratio of total to free serum cortisol</td>
<td>28</td>
<td>(none)</td>
<td>(28)</td>
</tr>
<tr>
<td>$s$</td>
<td>Supply factor of exogenous cortisol</td>
<td>1</td>
<td>nmol$^{-1}$ s$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$e_1(t)$</td>
<td>External input acting on plasma ACTH</td>
<td>Variable (dependent on time)</td>
<td>(none)</td>
<td></td>
</tr>
<tr>
<td>$e_2(t)$</td>
<td>External input acting on serum cortisol</td>
<td>Variable (dependent on time)</td>
<td>(none)</td>
<td></td>
</tr>
</tbody>
</table>

*In each subject who was experimentally studied, we applied the parameter identification method; for results, see Table 2. *Only in the data set on MR-blockade with cannoreat, we regard $MR_{\text{E}}^{\text{max}}$ and $GR_{\text{E}}^{\text{max}}$ as parameters, which have to be individually determined by the parameter identification method; for results, see Table 2. *The first parameter describes the combined effects of MRs in different parts of the brain, which are integrated into one final signal (acting on ACTH secretion). The same is true with the second parameter which describes the integrated GR effects. *To minimize the number of parameters, we used linear (i.e. non-sigmoid) dose-response relations for the effects at the adrenal and pituitary level. *Within this delay time, cortisol or ACTH are not active yet. *Used in the data-set on CRH-Stimulation ($e_1$ is a typical gamma distribution), in the other conditions $e = 0$. *Used in the data set on cortisol-infusion ($e_2$ is a ramp function), in the other conditions $e_2 = 0$. CORT, Cortisol; MR, mineralocorticoid receptor; CRH, corticotropin-releasing hormone.
Pharmacological blockade in healthy humans. We analyzed a data set derived from experiments using the MR antagonist canrenoate (96). When canrenoate was administered intravenously, serum cortisol concentrations were elevated compared with placebo (Fig. 3C, left). Similar results have been obtained in several other studies (3, 31, 35, 47, 99). Looking at the adrenal-to-brain feedback pathway, the fact that higher cortisol concentrations were observed suggests a different MR-GR feedback in the brain. Since canrenoate competes as a competitive antagonist with cortisol for binding to MR and (nonselectively to) GR, we identified the following parameters under canrenoate treatment: the affinities (described by $MR_{EC50}$ and $GR_{EC50}$) but not the efficacies ($MR_{E_{max}}$ and $GR_{E_{max}}$ brain)(Table 1). When the parameter identification procedure was applied to the experimental data, canrenoate was found to increase the $MR_{EC50}$ by around 7-

Table 2. Results from the parameter identification method

<table>
<thead>
<tr>
<th>Condition</th>
<th>$MR_{E_{max}}$</th>
<th>$GR_{E_{max}}$</th>
<th>$CRH_{E_{max}}$</th>
<th>$ACTH_{E_{max}}$</th>
<th>$GR_{E_{max}}$</th>
<th>$MR_{EC50}$</th>
<th>$GR_{EC50}$</th>
<th>Prediction Errora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women Normal</td>
<td>2.3 ± 0.1b</td>
<td>2.8 ± 0.1a</td>
<td>550 ± 50</td>
<td>79 ± 4</td>
<td>1.0 ± 0.1</td>
<td>h</td>
<td>i</td>
<td>0.046</td>
</tr>
<tr>
<td>Men Normal</td>
<td>2.0 ± 0.1b</td>
<td>3.2 ± 0.1a</td>
<td>440 ± 50</td>
<td>71 ± 4</td>
<td>1.2 ± 0.1</td>
<td>h</td>
<td>i</td>
<td>0.048</td>
</tr>
<tr>
<td>Men Placebo</td>
<td>f</td>
<td>f</td>
<td>f</td>
<td>f</td>
<td>0.7 ± 0.1</td>
<td>h</td>
<td>i</td>
<td>0.023</td>
</tr>
<tr>
<td>Men Canrenoate</td>
<td>f</td>
<td>f</td>
<td>f</td>
<td>f</td>
<td>0.5 ± 0.1</td>
<td>3.4 ± 0.8c</td>
<td>25.3 ± 4.1c</td>
<td>0.040</td>
</tr>
<tr>
<td>Addison’s disease</td>
<td>0.3 ± 0.2</td>
<td>3.1 ± 1.1</td>
<td>f</td>
<td>f</td>
<td>g</td>
<td>h</td>
<td>i</td>
<td>0.106</td>
</tr>
<tr>
<td>Cushing disease,</td>
<td>23.4 ± 6.3d</td>
<td>25.7 ± 6.5d</td>
<td>f</td>
<td>f</td>
<td>g</td>
<td>h</td>
<td>i</td>
<td>0.170</td>
</tr>
</tbody>
</table>

$^aP < 0.050$, men normal vs. women normal; ANOVA (women normal, men normal, and men obese) the other parameters as covariates. $^bP < 0.010$, men obese vs. men normal; ANOVA (women normal, men normal, and men obese) with the other parameters as covariates. $^cP < 0.005$, canrenoate vs. placebo; one sample $t$-test. $^dP < 0.005$. Cushing’s disease after adrenalectomy vs. Addison’s disease; independent Student’s $t$-test. $^e$Average prediction error based on distant function: (predicted value-observed value)/observed value. $^f$Adopted from normal men. $^g$Nonexistent in open-loop system, that is, in the absence of adrenal glands. $^h$MREC50 = 0.5 nmol/l is regarded as a constant, value taken from reference (Table 1). $^i$GREC50 = 5.0 nmol/l is regarded as a constant, value taken from reference (Table 1).

tive and negative feedback loops is consistent with the data set.

Pharmacological blockade in healthy humans. We analyzed a data set derived from experiments using the MR antagonist canrenoate (96). When canrenoate was administered intravenously, serum cortisol concentrations were elevated compared with placebo (Fig. 3C, left). Similar results have been obtained in several other studies (3, 31, 35, 47, 99). Looking at the adrenal-to-brain feedback pathway, the fact that higher cortisol concentrations were observed suggests a different MR-GR feedback in the brain. Since canrenoate competes as a competitive antagonist with cortisol for binding to MR and (nonselectively to) GR, we identified the following parameters under canrenoate treatment: the affinities (described by $MR_{EC50}$ and $GR_{EC50}$) but not the efficacies ($MR_{E_{max}}$ and $GR_{E_{max}}$ brain)(Table 1). When the parameter identification procedure was applied to the experimental data, canrenoate was found to increase the $MR_{EC50}$ by around 7-

Fig. 4. Effect of parameter-modifications on the prediction of ACTH and cortisol concentrations in healthy men. For validation of a model, it is mandatory to perform a sensitivity analysis (33, 34). Results of our sensitivity analysis are illustrated. A: stepwise increasing the values of the central parameter $MR_{E_{max}}$ by 125, 150, and 175%, leads to marked elevations of predicted ACTH and cortisol profiles. Thus, the predictions are very sensitive to changes in this parameter. B: using stepwise increased values of the central parameter $GR_{E_{max}}$, the predicted profiles of ACTH and cortisol are clearly lowered, indicating sensitivity to changes in this parameter. C: when the parameter $CRH_{E_{max}}$ is altered, we also find the predicted ACTH and cortisol profiles sensitively influenced. D and E: modifying the peripheral parameters $ACTH_{E_{max}}$ and $GR_{E_{max}}$ mainly affects the predicted cortisol profiles in a sensitive manner.

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Fig. 4. —Continued.
fold (P < 0.005), but the $GR_{E_{50}}$ only by around 5-fold (Table 2).

When the identified parameters were used to reconstruct the ACTH and cortisol profiles, these theoretic predictions were similar to the observed data (prediction error < 0.05; Fig. 3C, right, Table 2). When the identified parameters were used to analyze the brain corticosteroid feedback, the pharmacological MR blockade was plausibly found to shift the dose-response curve to the right (Fig. 3C, inset). Thus, we used the computational approach in the pharmacologically blocked HPA system and found results (i.e., increased EC$_{50}$ values for the corticoid receptors) that are in agreement with what is known about the characteristics of competitive blockade.

To interpret the influences of canrenoate, we refer to the three rules (Box 2). Rule 1: In the presence of competitive blockade (which affects MR and nonselectively also GR), cortisol binds only at medium concentrations to the brain MR and at very high concentrations to the brain GR (Fig. 6, top). Rule 2: Activated brain MR and GR operate in opposing manners. Rule 3: As long as brain MR are predominantly activated, a stimulatory effect prevails that acts to increase ACTH and consequently cortisol concentrations; as long as brain GR are predominantly activated, there is an inhibitory effect working that acts to decrease ACTH and so cortisol concentrations. In this way, the cortisol concentrations strive to a point of equilibrium at which the stimulatory and the inhibitory effects of the brain corticosteroid receptors are of the same magnitude (Fig. 6, bottom). These interactions would result in a homeostatic hypercortisolemic state under competitive blockade with canrenoate. On the basis of this reasoning, we can interpret that a blockade of the corticosteroid receptors elevates the brain setpoint of the HPA system (Fig. 3C inset); when cortisol has to compete with canrenoate for binding at the MR, it can only exert its full (MR-mediated) stimulatory drive in the HPA system at higher cortisol concentrations.

Looking in turn at the brain-to-adrenal forward pathway, the ACTH time courses were similar, but the cortisol concentrations were higher under canrenoate. We found canrenoate administration to decrease $GR_{E_{adrenal}}$, that is, efficacy of adrenal GR feedback. This finding is in line with the view of other researches suggesting that MR blockade affects the sensitivity of the adrenal gland to ACTH (99).

Genetic MR inactivation within the central nervous system represents another approach for testing our model of homeostasis. Central MR mutant mice show lower serum corticosteroid concentrations than their control littermates (10). We applied these published data in the parameter identification procedure and found a 20% (albeit not complete) reduction in the $MR_{E_{max}}^{brain}$. Merely, it remained unclear why the functional loss of MR efficacy was incomplete. Basically, our model of the HPA system also applies to this data set, in which the MR was genetically inactivated in the brain.

Experiments in patients with Addison’s and Cushing’s disease. We resorted to a data set that we had already published on patients with primary adrenal insufficiency, that is, Addison’s disease, and on patients with central Cushing’s disease, whereby the latter had had adrenalectomy (ADX) for therapeutic purposes (38). This experimental condition now made it possible to systematically investigate the brain corticosteroid feedback on ACTH almost over the entire range of cortisol concentrations, particularly focusing on the low-concentration range. When hydrocortisone was infused intravenously, the plasma ACTH in patients with Cushing’s disease/ADX was found to steeply rise in the beginning and then to continuously

Fig. 5. Effect of two-parameter-modifications on the precision of prediction in healthy men. Precision is determined by the deviation between two predictions: 1) the optimal prediction of data obtained in healthy men, 2) the prediction calculated with the modified parameters $MR_{E_{max}}^{brain}$ and $GR_{E_{max}}^{brain}$; high precision (red), low precision (blue). If the central parameters $MR_{E_{max}}^{brain}$ and $GR_{E_{max}}^{brain}$ are slightly modified, predictions markedly deviate from the optimal predictions in healthy men. Note that there is only one parameter set of $MR_{E_{max}}^{brain}$ and $GR_{E_{max}}^{brain}$ fulfilling the best model fit (dark red).
With the MR efficacy seen in patients with Addison’s disease/Cushing’s disease, the central MR efficacy is large compared to that seen in normal humans, when hydrocortisone (37) is administered. A similar initial and transient rise in plasma ACTH has been reported in normal humans, albeit less pronounced than in patients with Cushing’s disease. Of note, in a replication study on patients with Addison’s disease, we were able to detect an initial dose-dependent rise in plasma ACTH at low cortisol concentrations (see supplemental Fig. S1 online). In patients with Addison’s disease, low cortisol is the result of adrenal insufficiency. However, low cortisol, as it occurred after replacement therapy was withheld for 24 h, may also lead to secondary alterations in the MR:GR balance, as can be expected from experiments in adrenalectomized animals (69). We found that plasma ACTH continuously declined from the beginning of hydrocortisone infusion, thus indicating a dominant negative feedback effect of cortisol. Of note, in a replication study on patients with Addison’s disease, we were able to detect an initial dose-dependent rise in plasma ACTH (albeit less pronounced than in patients with Cushing’s disease) (37). A similar initial and transient rise in plasma ACTH has also been reported in normal humans, when hydrocortisone was infused during oral metyrapone treatment (97). With Cushing’s disease, the central MR efficacy is large compared with the MR efficacy seen in patients with Addison’s disease/ADX (23.4 ± 6.3 vs. 0.3 ± 0.2 fmol·l⁻¹·s⁻¹; P < 0.005; Table 2). Moreover, in patients with Cushing’s disease the “MR:GR balance” was markedly larger (1:1.09) than in normal men (1:1.60).

When the identified parameters were used to reconstruct the ACTH and cortisol profiles, the agreement between experimental data and theoretical prediction was satisfactory (prediction error <0.20; Fig. 3D, right, Table 2). When the identified parameters were used to analyze the brain corticosteroid feedback, central feedback effects over the entire cortisol range were found to be mainly inhibitory in patients with Addison’s disease and stimulatory in patients with Cushing’s disease (inset in Fig. 3D). Our model, therefore, adequately explains why the homeostatic level of cortisol is pathologically raised in Cushing’s disease.

Since some scientists have referred to experiments (3, 31, 35, 47, 73, 81, 89, 99) and concluded that the MR exerted a tonic inhibition at low cortisol concentrations, we tested the alternative system of differential equations that described an inhibitory feedback effect of MR, in addition to the inhibitory feedback of GR. When this alternative system was used to reconstruct the ACTH and cortisol profiles in Cushing’s disease/ADX, the observed peak ACTH concentration was found to deviate unacceptably from the prediction (prediction error > 0.60; see supplementary Fig. S2 online). Thus, our model had a wider scope of validity with the data sets examined here than a concept based on a MR tonic inhibition (Table 3).

Experiments in obese humans. Is the brain corticosteroid-feedback altered in obesity? When CRH was administered to obese male subjects (characteristics found in supplementary Table 1 online), ACTH responses were twofold higher, and the cortisol responses were slightly higher than in normal weight men (Fig. 3B, left). The obese men revealed a higher MR efficacy (2.4 ± 0.1 vs. 2.0 ± 0.1 fmol·l⁻¹·s⁻¹; P < 0.01) and a lower GR efficacy (2.8 ± 0.2 vs. 3.2 ± 0.1 fmol·l⁻¹·s⁻¹; P < 0.01) compared with the normal controls. In the obese men the “MR:GR balance” was larger (1:1.17) compared with healthy men (1:1.60). The peripheral adrenal parameters were of the same magnitude in the obese and control individuals.

The theoretical prediction shows a high agreement with the experimental observations (prediction error < 0.05; Fig. 3B right, Table 2). In obese men, central feedback inhibition was markedly reduced, especially at the high cortisol concentrations (inset in Fig. 3B). The computational approach allows locating specific derangements in the HPA system in all situations that are characterized by disturbed ACTH/cortisol secretion.

Table 3. Agreement, between two alternative models and different data sets

<table>
<thead>
<tr>
<th>Data sets</th>
<th>CRH challenge in healthy and obese humans, 50–500 nmol/l</th>
<th>Blockade with canrenoate in healthy humans, 130–500 nmol/l</th>
<th>Cortisol infusion in Addison and Cushing. ADX patients, 7–2000 nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>(cortisol range)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

*“A “pn-model”, as is proposed in the current paper, includes positive (p) MR mediated and negative (n) GR mediated. A “pn-model”, as is tested as an alternative model, includes negative MR (n) and negative GR (n) feedback loops. In case of agreement, we regard it dispensable to show the simulations. Note that this data set particularly covers the “MR-sensitive” low cortisol concentration range. See supplementary Figure S2. The scope of validity of the nn-model is smaller than that of the pn-model, as it is only in agreement with a subset of the tested data.

Fig. 6. Top: competitive blockade of the brain corticosteroid receptors and the right-shift of dose-response curves (horizontal arrows). Canrenoate predominantly affects the MR dose-response curve but also the GR dose-response curve in a nonselective manner (normal binding = solid lines; competitive blockade = dashed lines). In this way, the intersection (crossed circle) of the MR and GR receptor-kinetic curves is shifted to higher cortisol concentrations. Bottom: The summation curve of stimulatory MR and inhibitory GR feedback effects is also shifted to the right under competitive blockade with canrenoate. Note that canrenoate shifts the MR dose-response curve closer to the GR dose-response curve and in this way reduces the maximum of the biphasic feedback curve. The zero of the summation curve constitutes the brain setpoint of the HPA system (vertical arrows). At this setpoint, the brain’s stimulatory and inhibitory effects on cortisol secretion are of the same magnitude, and the HPA system stabilizes in a homeostatic state. Canrenoate can be viewed to shift the brain setpoint of the HPA system to higher cortisol concentrations.

GR: glucocorticoid receptor; MR: mineralocorticoid receptor.
One homeostatic system often conveys an input signal to another related homeostatic system in the organism. For example, energy homeostasis is closely linked to the HPA system (78). Our analysis shows that with obese men, the function of the GR is decreased and that as a result, the HPA system is less well-braked, that is, inhibited. The two stress systems, that is, the sympathetic nervous system and the HPA system, increase the energy supply of the brain. Recent studies have shown that in adrenalectomized animals the components of the HPA system are highly stimulated and that an exogenous application of glucose can almost completely return the hyperfunctioning HPA system to normal activity (8, 56, 57). Glucose can therefore substitute for the GR-mediated braking function of the HPA system (27). In a challenged organism, the alteration of one homeostatic system (e.g., brain energy metabolism) may strongly affect the homeostasis of another system (e.g., the HPA). Thus, input signals from related homeostatic systems can be influential, but, as we have demonstrated in this paper, they are not required to generate homeostasis.

DISCUSSION

The question addressed by this study was whether the presented “principle of homeostasis” (Box 2) is in agreement with data on the HPA system. The main finding of the study is that this model applies even for extreme conditions in the HPA system, like Addison’s and Cushing’s disease.

The discussion about homeostasis is closely linked to the question of how setpoints are generated in biological systems. In this paper, we showed that the HPA system can be described by a “pn-system”, that is, one that is driven by positive (p) MR-mediated and negative (n) GR-mediated feedback loops. We had also investigated an alternative “nn-system” that is driven by negative MR and negative GR feedback loops. The pn-systems are able to stabilize themselves with the help of a positive feedback loop to achieve equilibrium at a given setpoint. In contrast, the nn-systems do require a positive signal input into their regulatory loop; otherwise, they strive to equilibrium at zero values. Basically, generation of a setpoint requires a positive signal that originates either from the inside (positive feedback loop) or from the outside of a closed-loop system (external reference input). In humans, the HPA system is the major systemic stress system that normally stabilizes at a setpoint about midnight (ACTH 2 pmol/l, cortisol 50 nmol/l). It is important to maintain the low serum concentrations of cortisol during the slow wave sleep periods in the early night, because cortisol then predominantly binds to hippocampal MR, which is necessary for memory consolidation, and underlying synaptic plasticity in these neuronal networks (95). If the HPA system were an nn-system, it would require a steady positive signal input from outside the system (e.g., from the isocortex) to maintain midnight concentrations of ACTH and cortisol. If such a second system also were an nn-system, a third system would be necessary, and so on. Adding further nn-systems does not solve the problem of how homeostatic setpoints are created. From this theoretical point of view, homeostasis is possible only when at least one pn-system exists in the organism. Our data support the view that the HPA system itself is a required pn-system that allows achieving homeostasis at a given setpoint.

The problem that one experimental data set can be in agreement with two distinct theoretical approaches has often prompted a scientific debate. This issue is often referred to as “inverse problem” (87). Some researchers consider the HPA systems to be characterized by an nn-system, while we present the pn-system here. During the past decades, extensive empirical data accumulated that led researchers to assume an nn-system, which includes MR-mediated tonic inhibition, that is, negative feedback. The majority of these data has been obtained from experiments using more or less specific pharmacological MR blockade in humans (3, 31, 35, 47, 99) and animals (73, 81, 89). As a representative of such studies, we selected one from our own group for a more detailed mathematical investigation. In the original publication on the collected data (96), it was at first concluded that “MR blockade leads to a tonically increased cortisol secretion,” suggesting an nn-system regulation of cortisol. When the novel computational approach was used to reinvestigate the data, an nn-model was, in fact, found to fit these experimental data, but in contrast to some expectations, the pn-model, which included MR-mediated positive feedback, also fit the experimental data (Table 3). Such an ambiguity typically arises from an “inverse problem.” At this point, we emphasize that “MR-mediated tonic inhibition” is not necessarily the only explanation in this data set, but “MR-mediated positive feedback” is a plausible possibility. The above-mentioned data sets on carnoeanae cannot help us to distinguish which of the alternative models is more accurate in making predictions. Neither can the data sets on CRH challenge tests in healthy or obese humans help to make this distinction (Table 3). To solve an “inverse problem,” it might be helpful to investigate additional data sets. We therefore selected a data set that particularly focused on regulatory behavior of the HPA system in the low-cortisol concentration range in adrenalectomized patients. The comparison between the two alternative models shows that the pn-model fits with this data set, while an nn-model fails. Thus, we conclude that our model, which uses MR-mediated positive feedback components, has a wider scope of validity compared with a model with pure negative feedback (Table 3).

What are the underlying molecular mechanisms that could mediate positive and negative feedback in the HPA system? First, the brain mineralocorticoid and glucocorticoid receptors fulfill the key functions in the homeostatic rules (Box 2): binding cortisol with high and low affinities, acting in opposing manners, and mediating feedback effects on cortisol. The fact that the pair of MR and GR fulfills these required criteria supports MR and GR as one mechanism underlying positive and negative feedback in the HPA system, but we cannot rule out that other molecules are involved. Second, the fact that MR and GR fulfill the criteria (Box 2) does not allow us to infer whether genomic and nongenomic molecular mechanisms underlie the HPA system’s feedback. By genomic mechanisms, the corticosteroid receptors modify transcription of responsive genes, either through DNA binding or through protein-protein interactions with other transcription factors (7). Glucocorticoids influence de novo protein synthesis within a short, intermediate, and prolonged time-frame (41, 42). By nongenomic mechanisms, the glucocorticoids can exert fast effects on excitation-secretion coupling (48). Furthermore, MR were found indispensable for rapid nongenomic modulation of hippocampal glutamate transmission by corticosteroids (51). Cor-
corticosteroids also exert fast-onset inhibitory effects via membrane receptors (32, 93). There is the notion that the fast glucocorticoid actions, which are mediated by membrane receptors, are an ancient type of sterol/steroid-mediated effect and that!e may be the primordial glucocorticoid receptors (26). It is conceivable that brain corticosteroid feedback consists of a combination of genomic and nongenomic MR and GR actions. Whereas nongenomic MR and GR mechanisms modulate the early response, the late response is mainly influenced by genomic mechanisms, with the latter being more important in the homeostatic phase. Of note, the rules underlying our model (Box 2) do not include auxiliary conditions about time. Most relevant for our considerations are the balances assessed in the homeostatic state of equilibrium. Third, MR and GR display heterogenic distribution in the brain, and they may be located on excitatory or inhibitory neurons. Thus, a diversity of specific neuronal pathways and molecular mechanisms that underlye the brain corticosteroid feedback is conceivable. In all, on the basis of the current evidence, we regard MR and GR as the major mechanisms that fulfill the functions in the homeostatic rules and that underly brain corticosteroid feedback.

Is our model of the HPA system oversimplified? One point of view might be that important control mechanisms escape mention here so that the true complexity of the HPA system is not delved into. We have, in fact, refrained from including a large number of mechanisms that might be relevant in the regulation, for example, CBG, autoregulation of MR and GR and heterodimerization. First, CBG binds cortisol with high affinity but low capacity, so that the extent to which it binds cortisol saturates with midlevel cortisol concentrations. In this way, CBG acts like a functional buffer. When we tentatively included CBG in the model, we found that CBG had minor influence on the binding kinetics of MR and GR (particularly with rising or falling cortisol), but the crucial characteristics of two intersecting receptor-kinetic curves (Fig. 1D, top) were preserved. Second, autoregulation of MR and GR describes a short loop feedback process, by which both cerebral receptors influence their own gene expression. This process affects the efficacies of MR and GR. When we included autoregulation in the model, we observed that the system became more stable, but the essential features of two intersecting receptor-kinetic curves (Fig. 1D, top) remained conserved. Third, heterodimerization of MR and GR mainly affects how the separate receptor effects are integrated (61, 72, 88). When heterodimerization was included in a preliminary model (22), the combined feedback effect of both receptors effects (Fig. 1D, bottom) displayed an enhanced contrast between the positive and negative feedback effects, but again the critical aspects of the regulation remained unaffected. In all, there are several regulatory processes in the HPA system involved that might, of course, modify its regulatory behavior. Since using the presented minimal model of the HPA system led to satisfactory approximation in all of our data sets, we abstained from including extra biological mechanisms into the final model. We are aware that the restrictions proposed here might be the subject of debate, but we feel that the specifics of the model presented are less important than the general basic conception proposed here for homeostasis.

Our findings on the HPA system regulation lead us to speculate that the outlined principle also applies to other biological systems. Almost all known ligands bind to at least two receptors (a selection is shown in Table 4). In most of these dual receptor systems, the first and second rule (Box 2) has been demonstrated (17). In many dual-receptor systems, also the third rule has been established. These systems are the homeostatic ones. As a first example, the brain-derived neurotrophic factor (BDNF) binds to high- and low-affinity receptors and exerts counteracting effects on the survival and apoptosis of cells (Table 4). BDNF acts as an autocrine survival factor in neurons supporting its own release at low BDNF concentrations and inhibiting it at high concentrations. Thus, there is experimental evidence that all three homeostatic rules also apply to BDNF. As a second example, insulin has been shown to act by a dual receptor system with evidence for the existence of all three homeostatic rules (Table 4). The pancreatic β-cell secretes insulin molecules, which in an autocrine manner bind to high- or low-affinity insulin receptors located on the β-cell surface. Insulin acts on its own secretion either stimulatory at low insulin concentrations or inhibitory at high concentrations. In this way, insulin can stabilize its basal pancreatic secretion in a self-perpetuating process. As a third example, the three rules may also apply to the regulation of neuronal ATP (79). High- and low-affinity ATP-sensitive potassium channels adjust the excitation of neurons, the neuronal energy provision, and neuronal energy consumption. Here, dual ATP-sensitive

### Table 4. Examples of biological systems and respective evidence from the literature that the rules of homeostasis apply

<table>
<thead>
<tr>
<th>Receptor Class</th>
<th>Components of the Principle (Box 1)</th>
<th>Rule 1 References</th>
<th>Rule 2 References</th>
<th>Rule 3 References</th>
<th>Open-Loop B-Action</th>
<th>Closed-Loop B-Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear receptors</td>
<td>Cortisol MR GR</td>
<td>(28, 54, 82)</td>
<td>(12, 24, 50, 85)</td>
<td>*</td>
<td>LTP and cell-survival</td>
<td>† Cortisol</td>
</tr>
<tr>
<td>Tyrosine kinase</td>
<td>BDNF TrkB P75</td>
<td>(6, 19)</td>
<td>(11, 98, 100)</td>
<td>(1, 94)</td>
<td>LTP and cell-survival</td>
<td>† BDNF</td>
</tr>
<tr>
<td>Serine kinase</td>
<td>Insulin I-R IGF-1-R</td>
<td>(29, 83)</td>
<td>(59)</td>
<td>(55, 90, 101)</td>
<td>Insulin secretion</td>
<td>† Insulin</td>
</tr>
<tr>
<td></td>
<td>Leptin LR-L LR-S</td>
<td>(20, 66)</td>
<td>(5, 70)</td>
<td>Salience</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TGF b TGF-beta/ TGF-beta/</td>
<td>(40, 43)</td>
<td>(40, 43, 44)</td>
<td>Cell growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G protein coupled</td>
<td>Adrenaline β2</td>
<td>(68)</td>
<td>(16, 23, 58, 71)</td>
<td>(58)</td>
<td>Excitation</td>
<td></td>
</tr>
<tr>
<td>Mixed (G protein/channel)</td>
<td>Ach Nicotinic-R Muscarinic-R</td>
<td>(15, 62)</td>
<td>(60, 64, 65, 80)</td>
<td>Excitation</td>
<td>† Acetylcholine</td>
<td></td>
</tr>
<tr>
<td>KATP channel</td>
<td>ATP SUR1 SUR2</td>
<td>(49, 84)</td>
<td>(36, 86)</td>
<td>(79)</td>
<td>Excitation</td>
<td>† Neuronal ATP</td>
</tr>
</tbody>
</table>

*Shown in this paper to be in agreement with the regulation of cortisol. TGF, transforming growth factor; BDNF, brain-derived neurotrophic factor. I-R, insulin receptor; LR, leptin receptor; SUR, sulfonylurea receptor.
receptor systems exert a homeostatic influence on cellular energy metabolism. In summary, we can, therefore, find evidence for examples from most receptor classes, which suggest that many biological systems have evolved positive feedback loops to create homeostasis.

In this paper, we did not intend to show that the outlined model of the HPA-system regulation represents reality or that any other possibilities are excluded. We did demonstrate, however, that this model could fit all of our experimental data in a sensitive and robust manner and that among alternative models, the “principle of homeostasis” was in agreement with the largest data set, representing the widest scope of validity.

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