Supplementary Information

The Principle of Homeostasis in the Hypothalamus-Pituitary-Adrenal System: New Insight from Positive Feedback


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1. Supplementary Figures and Legends

Supplementary Figure S1. One-case replication study in a patient exhibiting central Cushing’s disease. By using up-to-date laboratory methods (see methods in the main paper) we replicated our former study (3), compared the new (orange data plot) with former time courses (grey data plot; mean ± 95%-confidence interval), and found stimulatory effect on ACTH at low cortisol concentrations confirmed.

Supplementary Figure S2. Alternative system of differential equations cannot predict data in Cushing’s disease. For experimental setting refer to supplementary methods below; data profile (black). We used an alternative system of differential equations that described inhibitory feedback effects of both MR and GR to reconstruct the ACTH and cortisol profiles (green). ACTH and cortisol profiles reconstructed by a system of differential equations based on the “principle of homeostasis” (orange).

Supplementary Figure S3. Total time lag in the brain corticosteroid feedback assessed by a simulation study. We used the differential equations (1) and (2) along with the parameters identified in the case C. H. (compare figure 2d) to simulate the cortisol and ACTH curves following a bolus injection of hydrocortisone (HC) at time 0. We found a 38-minute time interval between the cortisol peak and the consecutive ACTH nadir.
### 2. Supplementary Tables

**Supplementary Table 1. Characteristics of participants in the CRH challenge test**

<table>
<thead>
<tr>
<th></th>
<th>Age (a)</th>
<th>BMI (kg/m²)</th>
<th>Cortisol-binding globulin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women normal</strong></td>
<td>29.5 ± 2.1</td>
<td>22.3 ± 0.9</td>
<td>53.9 ± 7.3</td>
</tr>
<tr>
<td><strong>Men normal</strong></td>
<td>29.1 ± 4.4</td>
<td>23.4 ± 1.0</td>
<td>48.3 ± 2.6</td>
</tr>
<tr>
<td><strong>Men obese</strong></td>
<td>32.7 ± 2.6</td>
<td>37.0 ± 2.5</td>
<td>44.4 ± 2.2</td>
</tr>
</tbody>
</table>

P<0.01, men obese vs. men normal; independent student’s t-test
3. Supplementary Methods

*Pharmacological blockade in healthy humans; Suppl. Ref. (7)*

Twelve healthy nonsmoking, moderately trained, male volunteers participated in the study. Exclusion criteria were strenuous exercise within the preceding week, previous or present illness including psychiatric disorders, previous or current psychological stressors like upcoming exams, previous nighttime work, and any kind of medication at the time of the experiment. The local ethics committee approved the study, and all participants gave their written informed consent.

To evaluate the effect of a subchronic treatment, canrenoate (200 mg) or placebo was iv injected in two separate doses 24 and 8 h before the treadmill exercise after a randomized, double-blind, cross-over protocol. The two experimental sessions were divided by a washout period of 4–6 wk. On the experiment day, all participants were allowed to have a standard meal at 1200 h and drink plain water until the start of the experiment. The experiments started at 1515 h with the insertion of an iv catheter (Vasofix, Braun, Melsungen, Germany) into the antecubital vein of the right arm. At 1530 h participants rested comfortably on a bench in a supine position and blood samples for the determination of baseline values of ACTH, hGH, and cortisol were sampled every 20 min. After 60 min the participants started exercising on a treadmill (Jaeger, Hoechberg, Germany). To enable a steady physical stress over 1 h, the workload was set to 160 W. After a 60-min interval of exercise, participants were asked to rest comfortably in a horizontal position for the next 90 min. During the rest period, blood samples were taken every 20 min.

The data obtained in these experiments were reinvestigated by using our novel computational approach. For analysis, we only used data from the rest period.
Seven patients who had undergone total bilateral adrenalectomy for treatment of Cushing’s disease and four untreated patients with Cushing’s disease were studied. All examinations were performed in the morning hours, starting between 8 and 9 a.m.; replacement therapy was withheld for at least 24 hours. After an overnight fast an indwelling needle was placed into a forearm vein. Fifteen minutes later an infusion with 50 mg per hour of cortisol (50 mg of free alcohol in 20 ml of 50 per cent ethyl alcohol, 10 ml of this solution being diluted in 0.9 per cent sodium chloride to obtain a volume at 100 ml) was started and continued at constant rate for two hours with a Braun perfusor. Blood was drawn for plasma ACTH and plasma cortisol measurement at 15-minute intervals. We estimated plasma ACTH by radioimmunoassay after extraction of ACTH from the plasma as previously described (4; 6). We measured plasma cortisol by competitive protein-binding assay (5).

We compared the results with those obtained by the same procedures in patients with ACTH hypoadrenocorticism: four patients with primary adrenal insufficiency (Addison’s disease of one to 10 years’ duration) and three patients with congenital adrenal hyperplasia (21-hydroxylase deficiency). Four additional patients with Addison’s disease were subjected to the same procedure, but received the vehicle only (10 ml of 50 per cent ethyl alcohol in 90 ml of 0.9 per cent sodium chloride).

Of note, additional dose-response studies had demonstrated that the observed cortisol effects on the ACTH rise were in fact dose-dependent (1; 2).
Supplementary References


Figure S1

[Graph showing time in minutes on the x-axis and ACTH (pmol/l) on the y-axis. Two lines are plotted: one for the Former Study and another for M. Cushing/ADX Case U.J. with error bars indicating variability.]
Figure S2

Experimental data
Principle of homeostasis
Alternative model
Figure S3

ACTH (pmol/l)

0,0 0,5 1,0 1,5 2,0 2,5 3,0

time (min) -60 -30 0 30 60 90 120 150 180 210 240 270

Cortisol (nmol/l)

0,0 0,5 1,0 1,5 2,0 2,5 3,0

HC

Phase Shift