Neurobiology of Aging xxx (2017) 1–5

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Brief communication

Intranasal insulin decreases circulating cortisol concentrations during early sleep in elderly humans

Matthias Thienel [a], Ines Wilhelm [b], Christian Benedict [c], Jan Born [a,d,e], Manfred Hallschmid [a,d,e,*]

[a] Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Tübingen, Germany
[b] Child Development Center, University Children's Hospital Zurich, Zurich, Switzerland
[c] Department of Neuroscience, Uppsala University, Uppsala, Sweden
[d] German Center for Diabetes Research (DZD), Tübingen, Germany
[e] Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen (IDM), Tübingen, Germany

ARTICLE INFO

Article history:
Received 13 September 2016
Received in revised form 20 December 2016
Accepted 4 March 2017

Keywords:
Insulin
Brain
Sleep
Aging
Hypothalamic-pituitary-adrenal axis
Cortisol

ABSTRACT

Aging is associated with increases in hypothalamic-pituitary-adrenal (HPA) axis activity that can predispose to metabolic and cognitive impairments. We investigated in elderly and young subjects whether intranasal insulin administration to the human brain reduces early-sleep nadir concentrations of adrenocorticotropin and cortisol, that is, indicators of baseline HPA axis activity. In within-subject comparisons, intranasal insulin (160 IU) or placebo was administered to 14 elderly (mean age 70.0 years) and 30 young (23.6 years) healthy subjects before bedtime. Sleep was polysomnographically assessed and blood samples were repeatedly collected. Elderly compared with young participants displayed increased early-sleep cortisol concentrations ($p < 0.04$) and reductions in slow wave and REM sleep ($p < 0.001$). Insulin administration reduced cortisol levels between 2300 hours and 0020 hours in the elderly ($p = 0.03$) but not young participants ($p = 0.56$; $p = 0.003$ for interaction). Findings indicate that central nervous insulin acts as an inhibitory signal in basal HPA axis activity regulation and suggest that intranasal insulin may normalize sleep-associated stress axis activity in older age.

1. Introduction

The efficient regulation of neuroendocrine stress systems including the hypothalamic-pituitary-adrenal (HPA) axis is relevant not only with regard to their activation in response to environmental challenges but also to endogenous circadian rhythms. Release of adrenocorticotropin (ACTH) and cortisol is triggered by corticotropin-releasing hormone and regulated via glucocorticoid feedback at the hippocampal and hypothalamic levels. In the first night-half, ACTH and cortisol concentrations reach nadir values which indicate basal secretory HPA axis activity in the absence of external stimulation (Kern et al., 1996). Elderly humans display changes in sleep-related neuroendocrine patterns, in particular a decrease in growth hormone release and an increase in HPA axis activity, which are paralleled by a reduction in the amount of time spent in rapid eye-movement (REM) and slow-wave sleep (Ohayon et al., 2004; van Cauter et al., 2000). Since nocturnal hypercortisolism in aging individuals as a result of weakened central nervous control of HPA axis activity is assumed to contribute to disorders such as obesity, depression, and cognitive impairments (McEwen, 2000; Popp et al., 2015), identifying non-glucocorticoid factors that inhibit HPA axis activity may contribute to new approaches in the prevention and treatment of these ailments. Intranasal insulin, which is known to reach the brain compartment within 1 hour after administration (Born et al., 2002), has been repeatedly shown to attenuate HPA axis secretion (Benedict et al., 2004; Böhringer et al., 2008; Hallschmid et al., 2008). We investigated the effect of intranasal insulin on HPA axis secretion during early sleep in healthy elderly compared with young subjects. Considering the association between increased age and nocturnal HPA axis hyperactivity (van Cauter et al., 2000) as well as impaired central nervous insulin signaling (Biessels and Reagan, 2015), we expected an inhibitory effect of insulin on ACTH and cortisol secretion particularly in elderly subjects.

* Corresponding author at: Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Otfried-Müller-Str. 25, 72076 Tübingen, Germany. Tel.: +49-7071-2988925; fax: +49-7071-2925016.
E-mail address: manfred.hallschmid@uni-tuebingen.de (M. Hallschmid).

http://dx.doi.org/10.1016/j.neurobiolaging.2017.03.006

2. Materials and methods

2.1. Participants

Fourteen healthy elderly volunteers (8 men, 6 women; age, mean ± SEM, 70.00 ± 0.63 years; age range, 67–74 years; BMI, 24.83 ± 0.66 kgm^{-2}) and 30 healthy young individuals (16 men, 14 women; age, 23.63 ± 0.45 years; age range, 19–30 years; BMI, 22.93 ± 0.33 kgm^{-2}) participated in, respectively, experiments I and II. In the young participants, sleep-related electroencephalographic power spectra and memory performance were analyzed in addition and have been reported elsewhere (Feld et al., 2016). Relevant illness of our subjects was excluded by medical history and clinical examination. All subjects reported having a regular sleep-wake cycle and were not on medication except for estrogen-dominant oral contraceptives taken by all young women. All subjects spent 1 night in the sleep laboratory to adapt to the experimental procedure; visual inspection of respective polysomnographical results ensured that none of the subjects displayed abnormal sleep characteristics. They gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the local ethics committee.

2.2. Study design and procedure

Experiments followed a balanced, placebo-controlled, double-blind, within-subject, crossover design. All participants took part in 2 sessions which were identical except for the administration of insulin or placebo. Sessions were scheduled to be apart as close to 28 days as possible and the young women did not participate during their menstruation phases. Subjects were told to get up around 0700 hours and to abstain from naps or caffeine intake on experimental days, and to follow their usual dinner routines around 1800–1900 hours. Experiments started around 2000 hours. Electrodes were attached for standard polysomnographic recordings including electroencephalogram (at sites C3 and C4) that were scored offline according to standard criteria as wake, sleep stages N1, N2, N3, and REM sleep. At 2230 hours, subjects were intranasally administered a total dose of 1.6-mL insulin (160 IU; Insulin Actrapid; Novo Nordisk, Mainz, Germany) or vehicle (carrier solution) via sixteen 0.1-mL puffs (8 per each nostril) in 1-minute intervals. Subjects were allowed to sleep between 2300 hours (lights off) and 0700 hours (awakening), which corresponded to the period of polysomnographical recordings.

2.3. Blood sampling and control measures

Peripheral blood for the assessment of serum cortisol, C-peptide, insulin, as well as glucose and plasma ACTH was sampled during a pre-sleep baseline and at 20- to 40-min intervals during the first night-half until 0320 hours (see Fig. 1). For the group of elderly subjects, slight adjustments in the blood sampling schedule were introduced to increase the feasibility of repeated blood sampling and to restrict the burden of experimental participation, resulting in minor respective differences to the group of young subjects outside the main time period of interest (2300–0020 hours). Blood was drawn via long thin tubes enabling blood collection from an adjacent room while minimizing disruptive effects on the subject's sleep. Routine assays were used to determine concentrations of ACTH, cortisol, C-peptide (all Immulite, DPC, Los Angeles, CA, USA), insulin (Insulin ELISA Kit, Dako, Glostrup, Denmark), and glucose (HemoCue Glucose 201 Analyzer, HemoCue AB, Ångelholm, Sweden).

Appetite, thirst, and sleepiness were self-reported on visual analogue scales (0–100 mm) in both experiments. Mood, well-being, and subjective sleep quality were assessed via established rating scales, and heart rate and blood pressure were monitored before and after sleep.

2.4. Statistical analyses

For analysis of sleep stages, 1 female and 1 male participant of experiment II were excluded because of data loss. Analyses relied on Greenhouse-Geisser-corrected analyses of covariance for repeated measurements with baseline values as covariates and the between subject-factor 'sex' (male/female) and the within-subject factors 'treatment' (insulin/placebo) and 'time'. Areas under the curve (AUCs) were calculated according to the trapezoidal rule and single time points were compared by t tests. For comparisons between elderly and young subjects, linear mixed models were used with the between-subject factor 'age' (elderly/young). In addition, individual slope coefficients were obtained in the form of beta weights of linear regression lines fitted to ACTH and cortisol values between 2300 and 0320 hours and were compared between groups by 2-tailed unpaired t tests. A p-value <0.05 was considered significant; data are presented as means ± SEM.

3. Results

3.1. Increased HPA axis activity during early sleep in elderly compared with young subjects

Cortisol $AUC_{2300-0320\ h}$ values were higher in elderly compared with young subjects (13,472 ± 584 vs. 11,034 ± 972 nmol/L*min, $t_{(41)} = -2.22$, $p = 0.032$; $t_{(42)} = -0.74$, $p = 0.463$ for respective ACTH values). Accordingly, the increases in ACTH and cortisol concentrations emerging across the first night-halves of the respective placebo conditions were stronger in elderly than young subjects (beta weight means, ACTH, 0.15 ± 0.02 vs. 0.06 ± 0.01, $t_{(19)} = -3.48$, $p = 0.003$; cortisol, 6.81 ± 1.66 versus 0.64 ± 1.21, $t_{(41)} = -2.96$, $p = 0.005$). Nadir values of ACTH and cortisol did not differ between groups regarding levels (all $p > 0.20$) and timing ($p > 0.24$). Cortisol $AUC_{2300-0320\ h}$ values of the respective placebo conditions were moderately correlated with BMI in the elderly ($r = 0.54$, $p = 0.048$), but not in the young subjects ($r = -0.15$, $p = 0.43$).

3.2. Intranasal insulin dampens early-sleep cortisol concentrations in elderly subjects

Blood parameters did not differ between conditions during baseline (all $p \geq 0.15$). In the elderly subjects, insulin compared with placebo administration decreased cortisol concentrations during the first night-half (2300–0320 hours; $F_{(1,10)} = 5.83$, $p = 0.036$ for treatment; $t_{(13)} = 2.40$, $p = 0.03$ for the difference in $AUC_{2300-0020\ h}$), whereas this effect was absent in young participants (all $p > 0.44$; $F_{(22,129)} = 2.23$, $p = 0.003$ for treatment × time × age; Fig. 1A). In the elderly, the insulin-induced decrease in cortisol concentrations emerged irrespective of the subjects' sex ($p > 0.32$). Its extent was proportional to the respective cortisol nadir level in the placebo condition ($r = 0.60$, $p = 0.03$, Pearson's coefficient) but was statistically unrelated to changes in nocturnal levels of insulin, C-peptide, and glucose (all $p > 0.38$; $p > 0.46$ for the group of young subjects). Plasma ACTH levels were comparable between groups ($p = 0.13$) and were not influenced by treatment (both $p \geq 0.56$ for treatment; all $p \geq 0.10$ for single time point comparisons; Fig. 1B).

3.3. Serum insulin and blood glucose concentrations

Serum insulin concentrations were not affected by insulin administration in the elderly subjects (all $p \geq 0.58$). In the young