Brief communication

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

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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.

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2. Materials and methods

2.1. Participants

Fourteen healthy elderly volunteers (8 men, 6 women; age, mean $\pm$ SEM, 70.00 $\pm$ 0.63 years; age range, 67—74 years; BMI, 24.83 $\pm$ 0.66 kgm^{-2}) and 30 healthy young individuals (16 men, 14 women; age, 23.63 $\pm$ 0.45 years; age range, 19—30 years; BMI, 22.93 $\pm$ 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 $\pm$ 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 $\pm$ 584 vs. 11,034 $\pm$ 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 $\pm$ 0.02 vs. 0.06 $\pm$ 0.01, $t_{(19)} = -3.48$, $p = 0.003$; cortisol, 6.81 $\pm$ 1.66 versus 0.64 $\pm$ 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
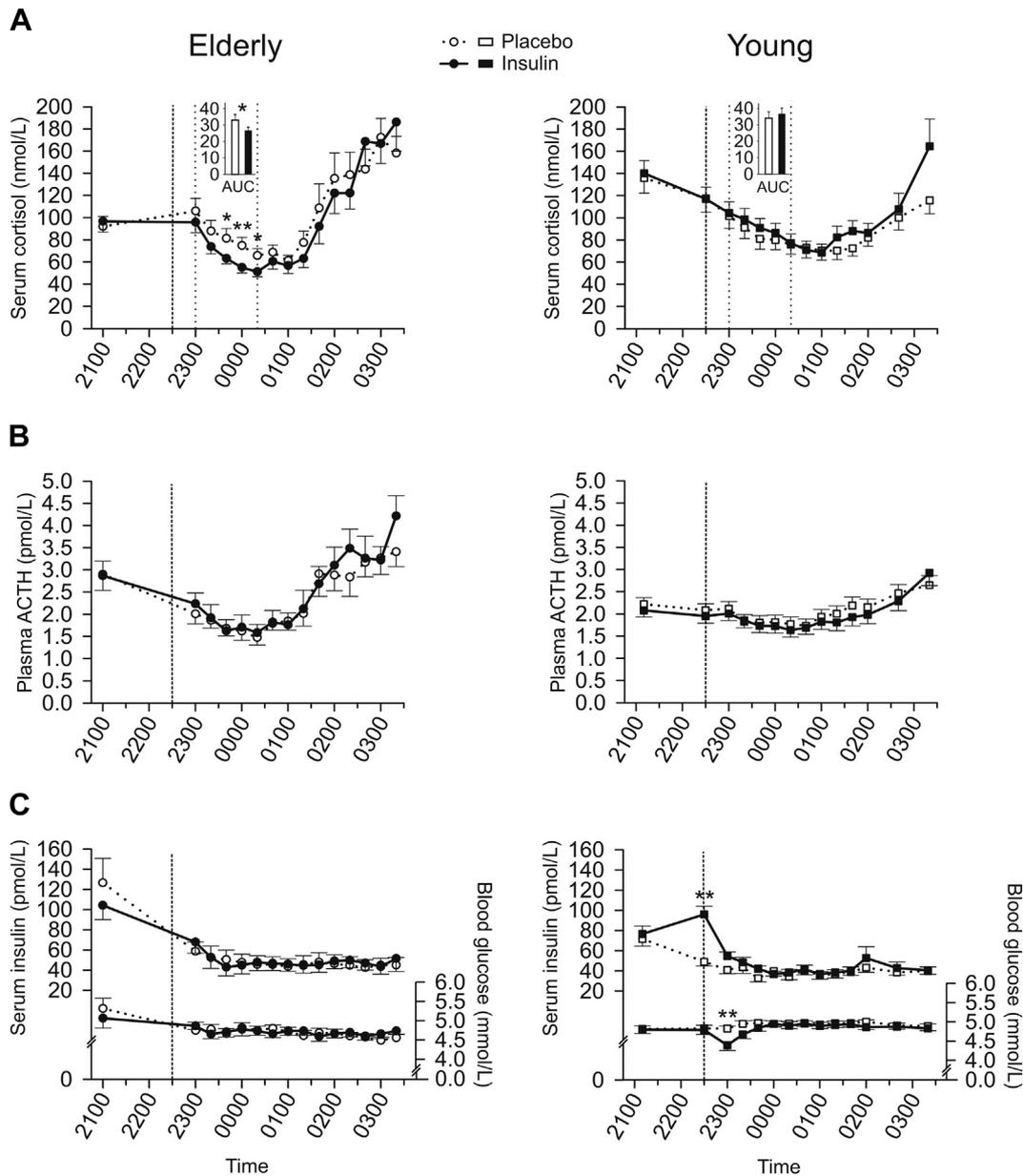
Fig. 1. Blood parameters. Mean ± SEM concentrations of serum cortisol (A; inserts depict $AUC_{2300-0020\ h}$ values in (nmol/L*min)/100), plasma ACTH (B), serum insulin and blood glucose (C; upper and lower lines, respectively) measured in elderly ($n = 14$; left panels) and young participants ($n = 30$; right panels) who were intranasally administered insulin (160 IU; black dots/squares/bars and solid lines) or placebo (white dots/squares/bars and dotted lines) at 2230 hours (dotted line). $^*p < 0.05$, $^{**}p < 0.01$ for comparisons between groups (t tests).

participants, they rose shortly after substance administration but were comparable between conditions thereafter ($p \geq 0.73$ for treatment × time; Fig. 1C, upper lines), with no statistical differences to the group of elderly subjects ($p = 0.24$ for age). In both groups, serum C-peptide concentrations slightly decreased after intranasal insulin administration (both $p < 0.1$ for differences at 2320 hours) but did not differ between conditions thereafter ($p = 0.68$ and $p = 0.85$, respectively, for treatment × time; $p > 0.62$ for age). In accordance with the ephemeral increase in peripheral insulin concentrations, in the group of young subjects blood glucose levels were acutely decreased after peptide administration at 2300 hours but subsequently returned to placebo condition values ($p = 0.65$ for treatment × time). Across conditions, blood glucose levels were lower in elderly than young individuals ($p < 0.001$ for age; Fig. 1C, lower lines).

3.4. Sleep parameters and control measures

Independent of the treatment, elderly in comparison to younger subjects had longer wake and light sleep (N1) periods at the expense of slow wave (N3) and REM sleep ($F_{(2,92)} = 29.78$, $p < 0.001$ for sleep stage × age; Table S1) assessed across the whole night. Sleepiness ratings and subjective estimations of sleep onset and sleep quality did not differ between groups (all $p \geq 0.20$). Intranasal insulin compared with placebo generally did not alter sleep latency, whole-night sleep architecture, and total sleep time (all $p > 0.29$). Early sleep (2300−0320 hours) likewise was unaffected by insulin in the elderly (all $p > 0.13$) and young subjects ($p > 0.20$), as was subjective sleep quality ($p > 0.53$; Table S1). Self-rated mood as well as hunger, thirst, and sleepiness ratings were not affected by insulin (all $p \geq 0.15$; see Table S1 for these and cardiovascular measures).

Elderly compared with young subjects showed a trend toward elevated systolic blood pressure values ($p = 0.07$ for age). Heart rate and blood pressure were generally not modulated by intranasal insulin (all $p \geq 0.18$).

4. Discussion

We demonstrate that intranasal insulin, which has been shown to reach the brain compartment (Born et al., 2002) and elicit functional brain effects (Hallschmid et al., 2004) within 1 hour after administration, reduces circulating concentrations of cortisol during the first night-half in elderly subjects. This result is in accordance with reports by our and other groups that intranasal insulin attenuates HPA axis activity in young men after long-term treatment (Benedict et al., 2004) and when administered before a social stress test (Böhringer et al., 2008). Physiologically, circulating insulin reaches the CNS via a receptor-mediated saturable transport across the blood-brain barrier and might dampen HPA axis secretion by acting on hypothalamic nuclei and limbic structures like the hippocampal formation, which express large numbers of insulin receptors (Plum et al., 2005). In contrast, euglycemic hyperinsulinemic clamps rather increase HPA axis secretion (Fruehwald-Schultes et al., 1999), probably by boosting hormone synthesis in the adrenal cortex that is not effectively reached by intranasal insulin. In comparison to their young controls, our elderly participants displayed increased secretory HPA axis activity as well as an increased amount of time spent awake and in sleep stage 1, but a decreased duration of REM sleep and sleep stage 3, indicators of decaying sleep quantity and quality generally associated with aging (van Cauter et al., 2000). Intranasal insulin did not induce significant changes in sleep architecture, which is in line with the lack of respective effects of peripheral hyperinsulinemia (Sturis et al., 1995). Although these observations suggest that insulin per se does not induce robust changes in sleep architecture, it cannot be excluded that higher doses of insulin or longer administration periods are necessary to modulate sleep quantity and quality in humans (Hallschmid et al., 2008; see Feld et al., 2016 for effects of intranasal insulin on electroencephalographic power spectra).

Intranasal insulin delivery reduced early-sleep cortisol concentrations in elderly but not in young participants. Importantly, sleepiness and subjectively experienced sleep onset and quality were comparable between groups, ruling out acute differences in psychological stress levels as mediators of the observed age-specific insulin effect. It is also unlikely that the small amount of intranasal insulin reaching the blood stream via spillover and causing a short drop in plasma glucose levels masked a centrally inhibiting insulin effect in the younger subjects by stimulating cortisol release. Spillover-induced increases in circulating insulin concentrations are negligible (Ott et al., 2015) compared to elevations needed to stimulate HPA axis activity under euglycemic conditions (Fruehwald-Schultes et al., 1999). Moreover, blood glucose levels remained clearly above the hypoglycemic threshold of 3.6–3.8 mmol/L where hormonal counterregulation sets in. The fact that changes in cortisol were generally unrelated to parameters of peripheral glucose homeostasis points to a central nervous mediation of insulin's suppressive effect on sleep-related cortisol concentrations in elderly subjects. We were not able to detect treatment effects on ACTH concentrations in the present study, so that an ACTH-independent mechanism of adrenal regulation may also be involved (Bornstein et al., 2008). This question clearly is in need of further clarification.

Our finding of an insulin effect in the elderly but not young subjects fits with previous studies indicating that in young, healthy men, attenuating effects of intranasal insulin on basal HPA axis activity only emerge after long-term administration (Benedict et al., 2004). Acutely attenuating effects of intranasal insulin on basal cortisol concentrations during wakefulness were found in obese (Hallschmid et al., 2008), but not in normal-weight men (Benedict et al., 2004). Obesity is associated with and also appears to be promoted by excessive HPA axis secretion, for example, due to chronic stress (Incollingo Rodriguez et al., 2015). Normal aging likewise goes along with alterations in the regulation of the HPA system, in particular increased nocturnal cortisol secretion (van Cauter et al., 2000). In our sample of elderly subjects, BMI was positively related to serum cortisol concentrations during the first night-half. In general, however, the elderly participants of the present study displayed a high level of physical health and only moderate signs of nocturnal HPA axis upregulation, which might explain the relative subtlety of the observed insulin effect. Still, the extent of insulin-induced cortisol reductions was associated with the height of respective cortisol nadir levels. Therefore, inhibitory effects of insulin on HPA axis secretory activity are expected to be stronger in aging subjects who show weaker nocturnal stress axis inhibition. In these subjects, intranasal insulin may be a helpful means to normalize nocturnal HPA axis activity and improve sleep-associated endocrine regulation. It remains to be seen if intensified or prolonged insulin administration paradigms may moreover induce associated changes in sleep parameters and, in this way, potentiate the beneficial metabolic and cognitive impact of central nervous insulin administration (Spetter and Hallschmid, 2015).

Disclosure statement

The authors have no actual or potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2017.03.006.

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