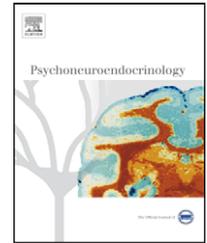




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# Intranasal insulin attenuates the hypothalamic–pituitary–adrenal axis response to psychosocial stress

Andreas Bohringer<sup>\*</sup>, Lars Schwabe, Steffen Richter, Hartmut Schachinger

Department of Clinical Physiology, University of Trier, Johanniterufer 15, 54290 Trier, Germany

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## KEYWORDS

Insulin;  
Intranasal administration;  
HPA axis;  
Cortisol;  
Psychosocial stress;  
TSST

**Summary** Previous studies have shown that intranasally administered insulin exerts an inhibitory influence on the basal hypothalamic–pituitary–adrenal (HPA) axis activity. To date, however, it remains unclear as to whether intranasal insulin does furthermore affect HPA axis responsiveness in situations of stress. Here, we tested whether intranasally administered insulin attenuates the HPA axis response to psychosocial stress.

Fifty minutes before being exposed to the Trier Social Stress Test (TSST), 26 healthy young male participants received a single intranasal dose of human insulin (40 I.U.) or placebo in a placebo controlled, double-blind between-subject design. Plasma cortisol, saliva cortisol, heart rate, and blood pressure were measured at resting baseline and in response to the TSST.

Plasma cortisol ( $P < .001$ ) and saliva cortisol ( $P < .001$ ) increased in response to stress, as did heart rate ( $P < .001$ ) and blood pressure ( $P < .001$ ). Intranasal insulin did not influence plasma or saliva cortisol, heart rate, blood pressure, blood glucose, and plasma insulin levels at baseline. However, intranasal insulin diminished the saliva cortisol (two-way ANOVA; treatment by time interaction:  $P = .05$ ) and plasma cortisol (two-way ANOVA; treatment by time interaction:  $P = .05$ ) response to the TSST without affecting heart rate, and blood pressure stress reactivity.

Our data show that a single intranasal insulin administration effectively lowers stress-induced HPA axis responsiveness. Intranasal insulin may offer a therapeutic potential to prevent hyperactivity of the HPA system.

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## 1. Introduction

Activation of the hypothalamic–pituitary–adrenal (HPA) axis is crucial for successful regulation of energy homeostasis during situations of stress (Sapolsky et al., 2000). However, hyperactivity of the HPA system is associated with several wide spread diseases like depression, arterial hypertension,

<sup>\*</sup> Corresponding author. Tel.: +49 651 201 3697;  
fax: +49 651 201 3737.

E-mail address: [boeh1303@uni-trier.de](mailto:boeh1303@uni-trier.de) (A. Bohringer).

visceral obesity, and the metabolic syndrome (Chrousos, 2000; Bjorntorp, 2001; Parker et al., 2003; Wirtz et al., 2006), where it contributes to the manifestation of these pathological states. To date our knowledge about the inhibitory control over the HPA axis activity is sparse and identification of factors that inhibit HPA axis activity may help to develop new therapeutic approaches against diseases characterized by HPA axis hyperactivity.

The pancreatic peptide hormone insulin plays a significant role in HPA axis regulation (Fruehwald-Schultes et al., 1999, 2001; Chan et al., 2005). Circulating insulin reaches the central nervous system (CNS) via a saturable active transport mechanism across the blood–brain barrier and binds to brain specific insulin receptors that are found with high density in hypothalamic nuclei and limbic structures (Unger et al., 1991; Plum et al., 2005). These brain structures are known to be involved in the regulation of HPA axis activity (Herman et al., 2005) and animal data indicate that insulin effects on the HPA axis are indeed mediated by actions on central nervous sites (Davis et al., 1995). In humans, intranasal insulin administration is an easy applicable tool for analyzing central nervous insulin effects (Fehm et al., 2000; Hallschmid et al., 2004). Intranasally administered insulin reaches the cerebro spinal fluid (CSF) without being absorbed into the blood stream (Born et al., 2002). Thus, this application method allows investigating central nervous insulin effects without confounding influences of peripheral insulin actions that are seen with systemic insulin infusions. Recently, it was shown that long-term treatment (8 weeks) with intranasally administered insulin reduces the morning HPA axis activity in lean (Benedict et al., 2004) and obese (Hallschmid et al., 2008) individuals and could thus offer a therapeutic way to treat hyperactivity of the HPA axis. Nevertheless, it remains unclear as to whether intranasally administered insulin may affect the HPA axis response to mental stress. This, however, would be of particular interest since human research revealed that HPA axis activation is closely linked to psychosocial challenge (Dickerson and Kemeny, 2004; Schwabe et al., 2008).

The present study examined the role of intranasally administered insulin on the HPA axis response to psychosocial stress. The Trier Social Stress Test (TSST) (Kirschbaum et al., 1993) was used as a psychosocially relevant stressor. This procedure is very effective in activating the HPA axis and has a straight forward relation to every day stress experiences (Dickerson and Kemeny, 2004). Changes in total plasma cortisol, saliva cortisol, heart rate, and blood pressure were measured as indices of HPA axis and cardiovascular responses to the stress challenge, respectively. Based on previous reports about inhibitory influences of intranasal insulin administration on the basal HPA axis activity (Benedict et al., 2004; Hallschmid et al., 2008) we hypothesized that intranasal insulin administration before TSST onset would attenuate the cortisol secretion in response to the stress challenge, as compared to placebo administration.

## 2. Subjects and methods

### 2.1. Participants

Twenty-six young, healthy male university students between 20 and 31 years of age participated in this study. Exclusion

criteria were as follows: any acute or chronic disease, smoking of cigarettes, familiarity with the TSST, a presence or history of mental illness, use of systemic medication, current participation in another clinical study, fasting glucose above 5.5 mmol/l, body mass index (BMI) below 18 or above 25, the presence of a depressive disorder screened with the German version of the Patient Health Questionnaire (PHQ-D, Loewe et al., 2002). Participants were required to fast for 6 h before arrival in our laboratory. All participants gave voluntary written informed consent and were compensated for their participation. The study was conducted in accordance with the declaration of Helsinki and was approved by the Ethical Committee of the State's Medical Association (Landesärztekammer Rheinland-Pfalz).

One participant of the insulin-group was excluded from further analyses because he showed baseline cortisol values that were two standard deviations above the average baseline cortisol values. Furthermore, one participant of the placebo-group was excluded because he did not meet exclusion criteria as turned out during the experiment.

### 2.2. Procedure

All participants arrived between 1330 h and 1530 h in our laboratory and were screened for exclusion criteria by the responsible physician. Participants were then randomly assigned to the insulin-group ( $n = 12$ ) or the placebo-group ( $n = 12$ ). Afterwards, a catheter (Vasofix, B. Braun, Melsungen, Germany) was inserted in an antecubital vein 105 min before start of the Trier Social Stress Test (TSST) to allow blood sampling at several time points across the experiment. Electrocardiogram (ECG) electrodes were attached according to a standard lead II configuration. The ECG was used for automated detection of heart rate. Fifty-five minutes before the stress challenge the first blood and the first saliva sample were obtained. Directly thereafter (50 min before TSST onset), either 0.4 ml (containing 40 I.U.) insulin (Actrapid<sup>®</sup> 100, Novo Nordisk) or a corresponding volume of placebo (dilution buffer without insulin; kindly provided by Dr. Manfred Hallschmid, University of Lübeck, Germany) were administered intranasally to the participants. The timing of intranasal insulin administration and the amount of 40 I.U. applied were chosen according to foregoing studies investigating acute effects of intranasal insulin on endocrine and cognitive parameters (Born et al., 2002; Hallschmid et al., 2008). Next, all participants drank 0.3 l of water in order to standardize the intake of liquid. Ten minutes prior to the TSST heart rate monitoring was started. In order to avoid influences of orthostatic reactions on heart rate changes during the TSST all participants were asked to change to a standing position before. Three minutes prior to the TSST the second blood-sample and the second saliva sample were collected and blood pressure was measured. The TSST was started for all participants between 1600 h and 1800 h. Immediately after its termination the third blood and saliva samples were obtained and blood pressure was measured again. Furthermore, participants evaluated on two 10-point rating-scales how stressful and insecure they felt during the TSST. Additional blood and saliva samples were collected 10, 20, 30, 45, 60, and 90 min after termination of the stress

test. Heart rate sampling was stopped 10 min after the end of the TSST. During the stay in our laboratory participants were not allowed to eat or drink anything. During the waiting periods between blood sampling they were obliged to restrict themselves to calm and non-arousing activities, such as reading newspapers.

### 2.3. The Trier Social Stress Test (TSST)

A detailed protocol of the TSST was described elsewhere (Kirschbaum et al., 1993). Briefly, the TSST is a standardized laboratory stressor consisting of a free speech and a mental arithmetic task in front of an audience and a video camera. Participants were introduced to the task and instructed to prepare a presentation in which they had to promote their candidacy for a job. After a 3-min preparation period, they were asked to give a 5-min free speech. Thereafter, participants were introduced to the mental arithmetic task, also standing in front of the audience. Subjects were required to count backwards from 2023 in steps of 17 as fast and accurate as possible for 5 min; upon a mistake they had to stop and start again at 2023.

### 2.4. Biochemical analyses

#### 2.4.1. Blood and saliva sampling

In order to determine the plasma cortisol, plasma insulin, and plasma glucose concentrations venous blood samples were collected in EDTA coated tubes (Monovette, Sarstedt, Germany). Samples were stored on ice for a maximum of 10 min and then centrifuged for 10 min at 6 °C 1200 × g. Plasma was stored at –20 °C until analyses of cortisol and insulin concentrations. Plasma glucose concentrations were determined prior to freezing. Saliva samples were collected in Eppendorf tubes (Eppendorf, Hamburg, Germany), stored at room temperature until completion of the session and then kept at –20 °C until analyses. After thawing for biochemical analyses, saliva samples were centrifuged at 2000 × g for 10 min.

#### 2.4.2. Cortisol

Total plasma cortisol was determined at all time-points of measurement with a commercial competitive enzyme amplified sensitivity immunoassay (ELISA, Immuno Biological Laboratories, IBL, Hamburg, Germany). Lower detection threshold of this assay was 6.9 ng/ml. The intra-assay variation ranged between 4.0% and 4.7%, the inter-assay variation between 5.0% and 9.6%. Saliva cortisol levels were determined employing a competitive solid phase time-resolved fluorescence immunoassay with fluoromeric end point detection (DELFI). This method was described in detail elsewhere (Dressendorfer et al., 1992). The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% and 9.0%.

#### 2.4.3. Glucose and insulin

Blood samples drawn 55 and 3 min prior to the stress challenge were used to analyze plasma insulin and glucose concentrations. Plasma glucose was determined by the hexokinase method (Olympus Analyzer, Olympus Life and Material Science

Europe GmbH, Hamburg, Germany). Plasma insulin concentrations were determined by an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). The lower detection threshold of this assay was 1.39 pmol/l. Inter-assay reproducibility ranged between 2.68% and 3.08%. The intra-assay precision was 0.93%.

### 2.5. Heart rates

Heart rate was derived from a single standard lead II ECG configuration employing telemetric HP 78100A transmitter and HP 78101A receiver system (Hewlett Packard Corp.). ECG was sampled by 1 kHz with 12 bit resolution. Beat detection was performed offline by WinCPRS (Absolute Aliens Oy, Turku, Finland) as was artifact control.

Heart rate measurements were taken continuously 10 min before, during, and 10 min after the TSST. The mean pre- and post-TSST heart rates as well as the mean task (preparation for speech, speech, arithmetic task) specific heart rates during the TSST were calculated for each participant.

### 2.6. Blood pressure

Blood pressure was measured 3 min before and 1 min after the TSST with a Criticon Dinamap device (SX1846 Dinamap Criticon, Tampa, FL).

### 2.7. Psychological assessment

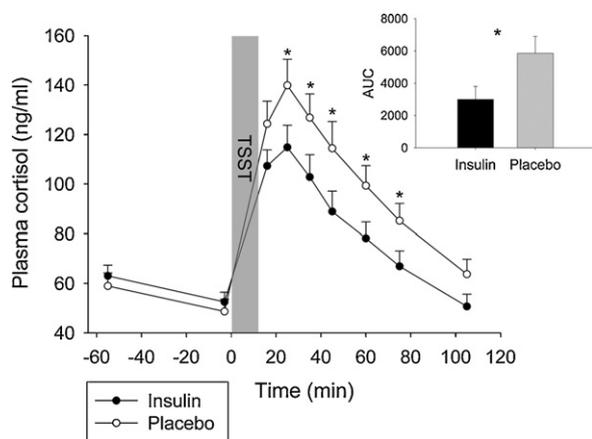
All participants rated on two rating scales ranging from 0 (not at all) to 10 (very much) how stressed and insecure they felt during the TSST.

### 2.8. Statistical analyses

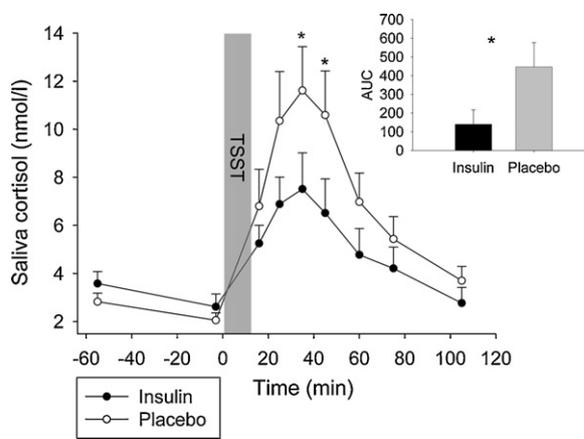
Data are presented as mean ± S.E.M. Kolmogorov–Smirnov tests revealed normal distribution for all variables. Effects of the pharmacological intervention as well as the stress challenge on endocrine, metabolic, and cardiovascular parameters were analyzed by two-way mixed design analyses of variance (ANOVAs) with the within-subject factor ‘time’ (timepoint of measurement) and the between-subject factor ‘treatment’ (insulin vs. placebo). Additionally, we calculated the area under the time response curve with respect to increase (AUC; Pruessner et al., 2003) according to the trapezoid rule for each participant and used one-way ANOVA to reveal influences of the pharmacological manipulation on the stress provoked cortisol secretion. The AUC was referenced to the cortisol concentration measured 55 min before TSST onset. Psychological variables were analyzed by one-way ANOVAs with the between-subject factor ‘treatment’ (insulin vs. placebo). Huynh-Feldt corrections were used for all analyses including repeated measures factors and only corrected results are shown. A *P* value ≤0.05 two-sided was considered significant.

#### 2.8.1. Missing-data

Due to technical error post-TSST blood pressure data of one participant in the placebo-group was lost.



**Figure 1** Mean ( $\pm$ S.E.M.) plasma cortisol concentrations before and after the TSST. Inset: mean ( $\pm$ S.E.M.) plasma cortisol AUC calculated in reference to the plasma cortisol value measured 55 min before TSST onset. The plasma cortisol AUC in the insulin-group (black bar) reached only 51% of the plasma cortisol AUC in the placebo-group (grey bar); comparisons at single time-points were done by means of one-way ANOVA; \* $P \leq .05$ .



**Figure 2** Mean ( $\pm$ S.E.M.) saliva cortisol concentrations before and after the TSST. Inset: mean ( $\pm$ S.E.M.) saliva cortisol AUC calculated in reference to the saliva cortisol value measured 55 min before TSST onset. The saliva cortisol AUC in the insulin-group (black bar) reached only 32% of the saliva cortisol AUC in the placebo-group (grey bar); comparisons at single time-points were done by means of one-way ANOVA; \* $P \leq .05$ .

### 3. Results

#### 3.1. Demographic variables

Both groups were comparable in age (insulin:  $24.2 \pm 0.9$  year; placebo:  $25.3 \pm 1.1$  year;  $F_{1,22} = .60$ ;  $P = .45$ ), weight (insulin-group:  $72.8 \pm 2.3$  kg; placebo-group:  $76.2 \pm 2.6$  kg;  $F_{1,22} = .94$ ;  $P = .34$ ), and body mass index (insulin-group:  $21.7 \pm 0.6$  kg/m<sup>2</sup>; placebo-group:  $22.5 \pm 0.5$  kg/m<sup>2</sup>;  $F_{1,22} = 1.3$ ;  $P = .28$ ).

#### 3.2. Pre-stress endocrine and metabolic measurements

The insulin and placebo groups did not differ in their plasma cortisol (TSST – 55 min:  $F_{1,22} = .36$ ;  $P = .56$ ; TSST – 3 min:  $F_{1,22} = .38$ ;  $P = .55$ ) and saliva cortisol (TSST – 55 min:  $F_{1,22} = 1.53$ ;  $P = .23$ ; TSST – 3 min:  $F_{1,22} = .84$ ;  $P = .37$ ) concentrations before onset of the TSST (Figs. 1 and 2, Table 1). Furthermore, groups showed comparable baseline glucose and insulin values (all  $ps > .57$ ; see Table 1). Whereas insulin ( $F_{1,22} = .55$ ;  $P = .46$ ) and glucose ( $F_{1,22} = .29$ ;  $P = .60$ ) values remained unchanged over time in both groups, plasma

cortisol ( $F_{1,22} = 16.98$ ;  $P < .001$ ;  $\eta^2 = .43$ ) and saliva cortisol ( $F_{1,22} = 11.70$ ;  $P = .002$ ;  $\eta^2 = .34$ ) concentrations declined within the pre-stress interval. This change in cortisol was comparable under both treatment conditions (*treatment* by *time* interaction; plasma cortisol:  $F_{1,22} < .01$ ;  $P = .97$ ; saliva cortisol  $F_{1,22} = .14$ ;  $P = .72$ ) indicating that the observed decline, most likely due to diurnal cortisol rhythmicity, was not affected by the insulin application. Thus, intranasal insulin administration did neither alter the circulating amount of insulin nor did it influence pre-stress cortisol, or blood glucose values.

#### 3.3. Endocrine, cardiovascular, and subjective responses to stress

##### 3.3.1. Cortisol

Both groups showed a strong increase in plasma cortisol ( $F_{2,1,47.6} = 75.18$ ;  $P < .001$ ;  $\eta^2 = .77$ ) and in saliva cortisol ( $F_{2,3,49.8} = 24.16$ ;  $P < .001$ ;  $\eta^2 = .52$ ) in response to the stress challenge, indicating that the TSST reliably activated the HPA axis (Figs. 1 and 2). Most relevant to the specific goals of the present study, significant *treatment* by *time* interactions revealed that the insulin and the placebo groups differed regarding their cortisol response to the stress test (plasma

**Table 1** Pre-stress saliva cortisol, plasma cortisol, plasma glucose and plasma insulin concentrations

	Time	Insulin-group	Placebo-group
Plasma cortisol (ng/ml)	TSST – 55 min	63.03 $\pm$ 4.34	58.95 $\pm$ 5.29
	TSST – 3 min	52.51 $\pm$ 3.93	48.65 $\pm$ 4.92
Saliva cortisol (nmol/l)	TSST – 55 min	3.57 $\pm$ .50	2.82 $\pm$ .35
	TSST – 3 min	2.61 $\pm$ .53	2.05 $\pm$ .31
Glucose (mmol/l)	TSST – 55 min	4.92 $\pm$ .10	4.99 $\pm$ .08
	TSST – 3 min	4.90 $\pm$ .08	4.89 $\pm$ .10
Insulin (pmol/l)	TSST – 55 min	26.50 $\pm$ 3.67	29.65 $\pm$ 3.81
	TSST – 3 min	27.85 $\pm$ 4.61	31.85 $\pm$ 5.39

Values are presented as mean  $\pm$  S.E.M.

**Table 2** Cardiovascular responses to the TSST

	Time	Insulin-group	Placebo-group
Heart rate (min <sup>-1</sup> )	Pre-TSST	71.8 ± 2.0	66.6 ± 2.8
	Preparation	83.1 ± 3.8	83.9 ± 5.5
	Speech	88.6 ± 5.4	92.7 ± 5.2
	Arithmetic	89.4 ± 3.8	89.2 ± 4.2
	Post-TSST	78.5 ± 3.4	74.1 ± 2.9
BP sys (mmHg)	TSST – 1 min	121.3 ± 3.1	124.3 ± 3.4
	TSST + 1 min	140.0 ± 3.9	146.2 ± 4.3
BP dia (mmHg)	TSST – 1 min	72.3 ± 2.5	75.1 ± 2.5
	TSST + 1 min	83.9 ± 2.5	86.2 ± 2.3

cortisol:  $F_{2,2,47.6} = 3.12$ ;  $P = .05$ ;  $\eta^2 = .13$ ; saliva cortisol:  $F_{2,2,49.8} = 2.90$ ;  $P = .05$ ;  $\eta^2 = .12$ ). One-way ANOVAs with the dependent variables plasma cortisol AUC and saliva cortisol AUC showed that both the plasma cortisol ( $F_{1,22} = 4.67$ ;  $P = .04$ ;  $\eta^2 = .18$ ) and saliva cortisol ( $F_{1,22} = 4.17$ ;  $P = .05$ ;  $\eta^2 = .16$ ) response to the TSST were significantly lower in the insulin-group as compared to the placebo-group. Insulin administration prior to the TSST reduced the mean plasma cortisol AUC by 49% (Fig. 1) and the mean saliva cortisol AUC by 68% (Fig. 2).

### 3.3.2. Heart rate and blood pressure

Changes in cardiovascular parameters in response to the TSST are summarized in Table 2. Both groups had comparable cardiovascular values prior to the stress test (heart rate:  $F_{1,22} = 2.36$ ;  $P = .14$ ; systolic blood pressure:  $F_{1,22} = .41$ ;  $P = .53$ ; diastolic blood pressure:  $F_{1,22} = .47$ ;  $P = .46$ ). Moreover, the TSST elicited a significant increase in heart rate and systolic as well as diastolic blood pressure in both treatment groups (all  $ps < .001$ ; all  $\eta^2 > .59$ ) indicating a cardiovascular stress reaction. However, in contrast to the observed effects on the HPA axis activity two-way ANOVAs revealed that the cardiovascular stress reaction was not influenced by intranasal insulin administration (*treatment* by *time* interactions and main effects *treatment* for heart rate and blood pressure data: all  $ps > .24$ ).

### 3.3.3. Psychological measures

All participants were asked to report on two 10-point rating scales ranging from 0 (not at all) to 10 (very much) how stressed and insecure they felt during the TSST. The two groups did not differ regarding their ratings of stressfulness (insulin-group:  $7.33 \pm .60$ ; placebo-group:  $6.92 \pm .54$ ;  $F_{1,22} = 0.26$ ;  $P < .61$ ) and insecurity (insulin-group:  $6.83 \pm .60$ ; placebo-group:  $5.50 \pm .53$ ;  $F_{1,22} = 2.77$ ;  $P = .11$ ). Since ratings of insecurity tended to be higher in the insulin-group as compared to the placebo-group we analyzed if differences in perceived insecurity mediated group differences in the stress provoked cortisol secretion. Controlling for insecurity by means of analyses of covariance (ANCOVAs) with cortisol concentration and AUC as dependent variable and insecurity as covariate did not change the results.

## 4. Discussion

The present study provides the first evidence that intranasally administered insulin attenuates the HPA axis response to

psychosocial stress in healthy young men. It is known that intranasal insulin reaches the CSF without entering the blood stream (Born et al., 2002) and circulating insulin levels were comparable among the insulin and the placebo-group before onset of the stress challenge in the current study. Thus, although peripheral insulin actions cannot be ruled out because we measured plasma insulin at two time points only, we suggest that the blunted HPA axis response to the TSST found in the insulin-group is most likely due to insulin effects on central nervous sites.

In line with our hypothesis we found an attenuating effect of intranasally administered insulin on the HPA axis response to psychosocial stress. This result extends previous reports about inhibitory influences of intranasally administered insulin on the basal HPA axis activity (Benedict et al., 2004; Hallschmid et al., 2008) to the domain of stress related HPA axis activity. Previous studies that investigated effects of insulin on the stress-induced HPA axis activity focused on physiological stressors solely. Most of them examined the effects of hypoglycemia stress at different levels of systemic hyperinsulinemia. These studies provided rather inconsistent results. Some authors reported enhancing (Davis et al., 1993; Lingenfelser et al., 1996) others attenuating (Kerr et al., 1991) or no effects (Diamond et al., 1991; Fisher et al., 2005) of insulin on the HPA axis response to hypoglycemia. This discrepancy might be due to differences in sample characteristics (e.g. testing healthy subjects or participants with insulin dependent diabetes). One study investigated effects of brain insulin signaling on the HPA axis response to hypoglycemia stress (Davis et al., 1995). The authors found that a selective increase in the level of insulin in the blood perfusing the brain enhances the cortisol response to hypoglycemic stress in dogs, as compared to peripheral insulin infusion. This suggests a stimulatory effect of insulin on the HPA axis response to hypoglycemia at the CNS level. In the present study, however, we obtained an attenuated HPA axis response to psychosocial stress following administration of intranasal (i.e. centrally acting) insulin. This discrepancy in CNS insulin effects on the HPA axis might be owing to the different species studied or differences in the way of insulin delivery. Furthermore, the obviously diverging insulin effects may be explained by the different stressors used, i.e. physiological/ metabolic vs. mental stressors. Importantly, it was suggested that HPA axis reactions to simple systemic stressors like hypoglycemia rely crucially on brainstem and direct systemic projections to the hypothalamus (Pacak and Palkovits, 2001). In contrast, stressors requiring interpretative processing like psychological stress tests involve an activation of limbic and

higher-order brain structures (Herman and Cullinan, 1997; Herman et al., 2005). Thus, specific insulin actions on brain-stem, limbic, and higher-order brain structures may account for diverging insulin effects on the HPA axis response to physiological stressors like hypoglycemia and psychological stressors like the TSST.

Limbic structures, particularly the hippocampus and the amygdala, have been shown to play an important modulatory role in HPA axis regulation with the hippocampus having inhibitory and the amygdala having excitatory influences (Herman et al., 2005). Interestingly, both structures express insulin receptors (IRs) at a high density (Unger et al., 1991). It is tempting to speculate that insulin exerts its modulating effect on the HPA axis via its influence on hippocampal or amygdaloid neurons. Furthermore, it is well known that insulin has profound effects on hypothalamic nuclei involved in the regulation of energy homeostasis (Benoit et al., 2004; Niswender et al., 2004; Plum et al., 2005). In particular, neurons within the arcuate nucleus (ARC) of the hypothalamus are affected by insulin (Schwartz et al., 1992; Benoit et al., 2002). Since it was shown that the ARC is crucially involved in normal regulation of HPA axis activity (Bell et al., 2000) modulation of ARC neurons could be another route of insulin action on the HPA axis.

Intranasal insulin administration did not alter the basal HPA axis activity in the present study, and the plasma and saliva cortisol concentrations declined comparably in the insulin and the placebo-group over the pre-stress interval. While this finding is in line with a previous study that revealed attenuating effects of chronic but not of acute intranasal insulin treatment on the HPA axis activity (Benedict et al., 2004) another study suggests that intranasal insulin administration may acutely decrease the circulating amount of cortisol (Hallschmid et al., 2008). In contrast to this attenuating effect of intranasally administered insulin on the basal HPA axis activity, other studies showed that high levels of systemic hyperinsulinemia during euglycemic glucose clamps could directly activate the HPA axis secretory activity in rats (Chan et al., 2005) and humans (Fruehwald-Schultes et al., 1999, 2001). The discrepancy between studies involving intranasal insulin administration and hyperinsulinemic glucose clamp techniques may be explained by diverging insulin effects at central and peripheral levels of the HPA axis. Importantly, results from an *in vitro* study suggest that systemic hyperinsulinemia may affect the steroid hormone synthesis in adrenal cells that are not reached by intranasal insulin (Penhoat et al., 1988).

Here, we did not find effects of intranasal insulin on the cardiovascular stress reaction. Such dissociations between the HPA axis stress reactivity and markers of autonomic arousal have previously been reported (Kirschbaum et al., 1993, 1997; Schommer et al., 2003; Fries et al., 2006). Intranasally administered insulin appears to be another modulator of stress reactivity that influences specifically the endocrine stress reaction without having effects on corresponding autonomic markers and cardiovascular parameters.

Some limitations of the present study have to be discussed. First, we focused on male participants only since it is known that estradiol and progestins modify the endocrine stress reaction to psychosocial stress (Kirschbaum et al., 1999). Further studies will have to corroborate our findings in women. Second, our study was based upon a rather small

sample size. Future studies involving a bigger sample size may offer the opportunity to investigate influences of intranasal insulin on the endocrine stress reaction and subjective responses to psychosocial stress in more detail.

In conclusion, the present study demonstrates that intranasal insulin attenuates the HPA axis response to psychosocial stress in healthy male subjects. This finding points to a modulatory role of brain insulin signaling in the regulation of HPA axis activity. Furthermore, our data suggest that the intranasal route of insulin delivery may offer a new therapeutic approach to prevent increased excitability of the HPA system.

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## Conflict of interest

All authors have nothing to declare.

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