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Intranasal insulin improves memory in humans

Christian Benedict[a,*], Manfred Hallschmid[a], Astrid Hatke[a], Bernd Schultes[b], Horst L. Fehm[b], Jan Born[a], Werner Kern[b]

[a]*Department of Neuroendocrinology, University of Lübeck, Ratzeburger Allee 160, Haus 23a, 23538 Lübeck, Germany*
[b]*Department of Internal Medicine I, University of Lübeck, Ratzeburger Allee 160, Haus 23a, 23538 Lübeck, Germany*

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Insulin; Memory; Intranasal administration; Mood

Summary Previous studies have suggested an acutely improving effect of insulin on memory function. To study changes in memory associated with a prolonged increase in brain insulin activity in humans, here we used the intranasal route of insulin administration known to provide direct access of the substance to the cerebrospinal fluid compartment. Based on previous results indicating a prevalence of insulin receptors in limbic and hippocampal regions as well as improvements in memory with systemic insulin administration, we expected that intranasal administration of insulin improves primarily hippocampus dependent declaration memory function. Also, improvements in mood were expected. We investigated the effects of 8 weeks of intranasal administration of insulin (human regular insulin 4 x 40 IU/d) on declarative memory (immediate and delayed recall of word lists), attention (Stroop test), and mood in 38 healthy subjects (24 males) in a double blind, between-subject comparison. Blood glucose and plasma insulin levels did not differ between the placebo and insulin conditions. Delayed recall of words significantly improved after 8 weeks of intranasal insulin administration (words recalled, Placebo 2.92 ± 1.00, Insulin 6.20 ± 1.03, $p < 0.05$). Moreover, subjects after insulin reported signs of enhanced mood, such as reduced anger ($p < 0.02$) and enhanced self-confidence ($p < 0.03$). Results indicate a direct action of prolonged intranasal administration of insulin on brain functions, improving memory and mood in the absence of systemic side effects. These findings could be of relevance for the treatment of patients with memory disorders like in Alzheimer's disease.

1. Introduction

Recent studies have suggested a role for insulin not only in controlling systemic blood glucose concentrations but also in influencing various central nervous functions. So far two main roles of insulin in the brain have been described: on the one hand, insulin affects hypothalamic structures involved in body weight regulation (Porte and Woods, 1981), on the other hand, it influences memory processing (Marfaing et al., 1990; Park et al., 2000; Kern et al., 2001). The neuroendocrine effect of insulin on memory may involve

*Corresponding author: Tel.: +49-451-500-4602; fax: +49-451-500-3640.
E-mail address: benedict@kfg.uni-luebeck.de (C. Benedict).

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brain insulin receptors which are predominantly located in the hippocampus and connected limbic brain structures (Unger et al., 1991; Lannert and Hoyer, 1998). These brain areas are essential for declarative memory formation (Squire, 1992; Eichenbaum, 2001). Changes in declarative memory after insulin administration have been investigated both in animals and humans. In rats, intracerebroventricularly administered insulin improved memory for a passive avoidance task (Park et al., 2000). In humans, intravenous infusion of insulin during euglycemic conditions was found to improve especially hippocampus-dependent types of declarative memory (Kern et al., 2001). However, intravenously administered insulin is unsuitable for long-term treatment of memory since it causes side effects like hypoglycemia and changes in plasma ionic balances. A route of insulin intake avoiding these peripheral side effects is the intranasal administration (Born et al., 2002). Substances such as insulin after intranasal administration are considered to enter the cerebrospinal fluid from the nasal mucosa via intercellular clefts along the nervus olfactorius and the bulbus olfactorius (Illum, 2000). The transport of peptides and small proteins from the nose to the cerebrospinal fluid has been consistently observed in many previous studies before (Balin et al., 1986; Sakane et al., 1991). The positive findings of improved memory following intravenous administration of insulin in combination with the high density of insulin receptors found in limbic and hippocampal brain regions led us to assume that intranasal treatment with insulin induces improvements particularly in hippocampus dependent types of declarative memory. Also, in light of previous improvements in mood (Kern et al., 2001) seen in humans treated with intravenously administered insulin, we expected that intranasal insulin administration could cause similar improvements in mood.

2. Materials and methods

2.1. Subjects, design and procedure

38 students (24 males; 18–34 yrs) of normal body weight and without personal or family history of diabetes were examined. Isle cell antibodies and fasting plasma glucose were determined in blood to exclude diabetes. Also, subjects underwent a physical examination to ensure they were healthy. Ten hours prior to testing they had to fast and to abstain from coffee and alcoholic beverages. The study was approved by the local Ethics Committee on Research Involving Human Subjects, and written informed consent was obtained from all subjects.

Subjects were randomly assigned to two groups (each 12 males, 7 females) which were comparable for age (Insulin: 25.26 yrs $\pm$ 1.21, Placebo: 25.63 yrs $\pm$ 1.25) and body mass index (Insulin: 22.6 $\pm$ 0.3 kg/m^2; Placebo: 22.7 $\pm$ 0.4 kg/m^2). During a two-week baseline phase, all subjects received placebo, during the following eight-week treatment phase subjects were intranasally administered either insulin or placebo. Substances were administered four times a day: in the morning, around noon, in the evening (approximately half an hour before mealtime, respectively), and before going to bed. Each dose consisted of 0.4 ml insulin (containing 40 IU; Insulin Actrapid® HM, Novo Nordisk, Mainz, Germany) or vehicle (HOE 31 dilution buffer for H-Insulin, Aventis Pharma, Bad Soden, Germany) administered within 4 puffs of 0.1 ml (2 per nostril), amounting to 1.6 ml (160 IU) insulin or vehicle per day. The daily dose of insulin was based on previous results demonstrating in humans a nearly two-fold increase in cerebrospinal fluid concentration of insulin within 120 min after the intranasal administration of the hormone at a single dose of 40 IU. Accordingly, the dose of the study was expected to induce temporary increases in cerebrospinal fluid concentrations of insulin distinctly above the normal concentration in healthy lean individuals. Note also, that with intranasal administration the doses used are much higher than with subcutaneous and intravenous modes of administration, since after intranasal administration much of the substance just passes through the nose and pharynx and is not absorbed. Sprays were stored in a refrigerator at about 4 °C and were replaced by new substance every 7 days. In order to assure compliance, subjects kept a protocol on their intake routine and they were told that irregular intake would be detected via urine sampling. Test sessions, which were scheduled at 0800 h in the morning, took place at the beginning of the baseline phase, at the start of the treatment phase and one week before its end (Fig. 1a). Sessions at the start of the treatment phase were conducted 60 min after administration of an initial dose of 40 IU insulin or placebo in order to examine acute effects of intranasal insulin administration. Long-term effects were measured in the session one week before treatment ended. Here, all subjects received placebo spray 60 min before testing to exclude acute insulin influences. To test long-term memory recall, additional sessions took place one week after the above described sessions, i.e., after one week of the baseline phase and after one week and 8 weeks of insulin treatment. Here, subjects also received placebo in

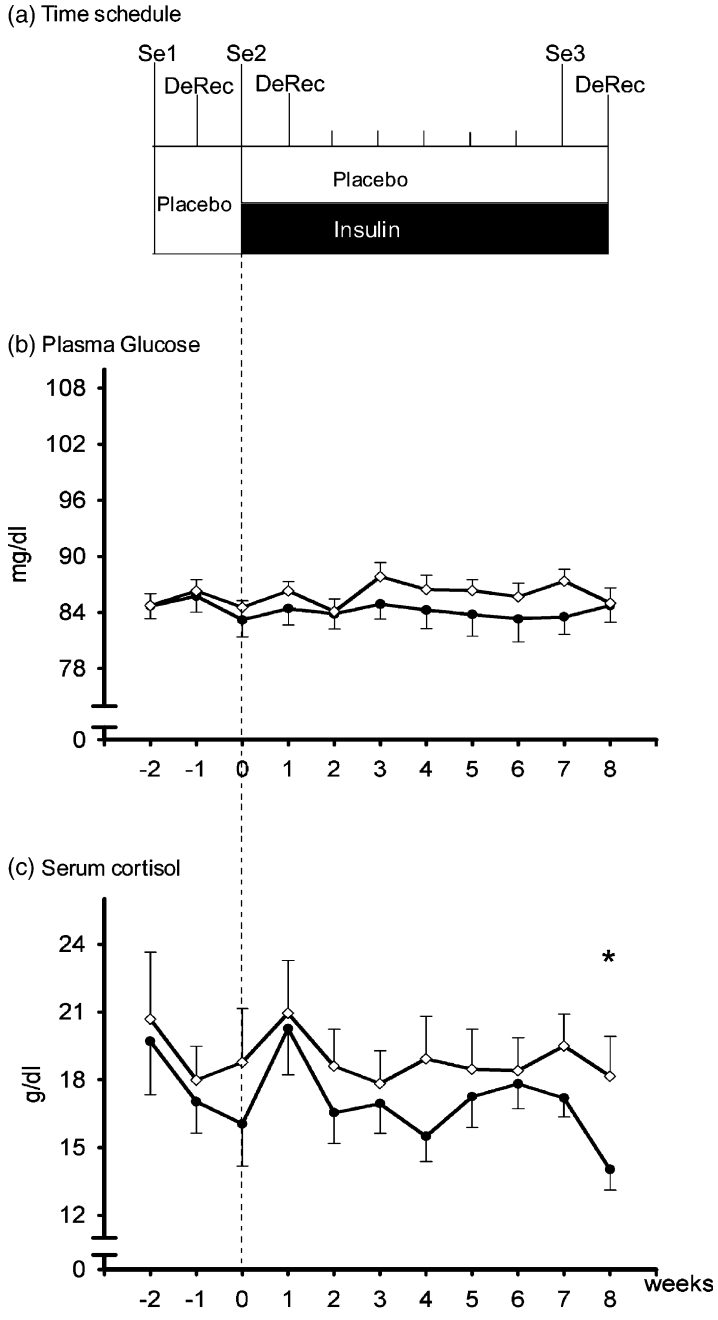

Fig. 1. (a) Two groups of 19 subjects each were intranasally treated with placebo for two weeks. Then, one group received insulin. Intranasal treatments were performed 4 times per day (160 IU Insulin/d). Test session took place in the beginning of the study (session 1, Se1), after the first intranasal administration of insulin (session 2, Se2) and in the seventh week of the treatment period (session 3, Se3). Delayed recall of words learned in each of these sessions was tested one week later (DeRec), respectively. (b) Mean (± SEM) plasma glucose concentrations before and during subchronic (8 weeks) intranasal treatment with insulin (-●-, 4×40 IU/d) and placebo (-□-). Blood was sampled once a week (between 0800 and 0900 h). Values are baseline-adjusted as derived from ANCOVA, $*p < 0.05$. (c) Mean (± SEM) serum cortisol concentrations before and during subchronic (8 weeks) intranasal treatment with insulin (-●-, 4×40 IU/d) and placebo (-□-). Blood was sampled once a week (between 0800 and 0900 h). Values are baseline-adjusted as derived from ANCOVA, $*p < 0.05$.

both groups 60 min before testing. Blood samples for determination of cortisol (Cortisol-RIA, DPC Biermann GmbH, Bad Nauheim, Germany), insulin (Pharmacia Insulin RIA100, Pharmacia & Upjohn, Inc., Uppsala, Sweden), and blood glucose (by the hexokinase method; Abbott; Wiesbaden) were collected in the end of each test session, and on a weekly basis between experimental sessions.

Additionally, epinephrine and norepinephrine in 12 h nocturnal collection (2000 – 0800 h) urine were measured weekly by high performance liquid chromatography (Watchs Co., Milford, MA).

2.2. Memory tasks, cognitive tests, and mood assessment

2.2.1. Word list

In this declarative memory test, a list of 30 words was presented. The words belonged to three semantic categories, neutral (e.g., 'tree', 'field'), food-related (e.g., 'ham', 'eggs'), and emotional (e.g., 'mother', 'friend'), and were presented orally at a rate of 1 word/sec. Subsequently, subjects were told to remain silent for a break of 3 min and to keep the presented words in mind. For immediate recall, subjects wrote down all words they remembered within 90 sec. For delayed recall, approximately one week later, subjects again had to write down all words they still remembered from this list (Fruehwald-Schultes et al., 2000; Kern et al., 2001; Greenwood et al., 2003). Note, that the study design did not allow for testing acute effects on delayed recall, since once having started, treatment was continued for 8 weeks including also both 1 week retention intervals for delayed recall. In a short post-treatment interview, none of the subjects stated to have learned or thought about the word list within the week before delayed recall, excluding any interference with rehearsal effects.

2.2.2. Wordstem priming

Nondeclarative memory was tested with a wordstem priming task based on a learning word list and a test list of two-letter wordstems. First, subjects rated the nouns of the learning word list according to their sound on a 5-point scale (from 1 = unpleasant to 5 = pleasant). This task was considered to induce implicit learning. Thereafter the subjects received the test list containing 52 two-letter wordstems (e.g. ''ho'' derived from''-hotel''). Twenty-six wordstems of this list were derived from the (rated) learning list, whereas the other 26 wordstems were taken from a pool of new words not presented to the subject (new list). Subjects were instructed to complete the wordstems to the first noun that came to their mind. The difference between the number of wordstems ''correctly'' completed to nouns from the learning list and the number of words accidentally completed to nouns of the new list was considered a measure of implicit memory (Plihal and Born, 1999a).

2.2.3. Stroop test

The Stroop test included three subtests, the word reading test, the color naming test, and the interference test. On the reading test, subjects were presented a panel with a series of color names (green, red, blue, yellow) printed in black ink (word reading subtest). On the color naming test, subjects were presented rows of Xs in different colors (color naming test). Subjects respectively read the words and named the colors as quickly as possible. The interference subtest is considered a measure of selective attention (Golden, 1978). Here, subjects were presented a series of color names (green, red, blue, yellow) printed in different colors. Their task was to name as quickly as possible the color of the ink in which each word was printed, but to inhibit the prepotent reading response. For each test, the total number of correct responses within 45 seconds was determined. The presentation of the word list learning task, the wordstem priming task, and the Stroop test was balanced across subjects.

2.2.4. Mood assessment

In the end of each test session subjects filled in an adjective check list designed to assess actual mood and feelings of activation on 15 dimensions. The adjective checklist consists of a total of 161 adjectives used to describe the subject's mood on 15 dimensions: (translated from German) ''Activation'', ''Concentration'', ''Deactivation'', ''Fatigue'', ''Benumbed'', ''Extraversion'', ''Introversion'', ''Self-confidence'', ''Well-being'', ''Arousal'', ''Sensitiveness'', ''Anger'', ''Anxiety'', ''Sadness'', and ''Dreaminess''. For each adjective, the subject had to indicate whether or not it reflected aspects of his/her current state of mood. For each dimension, the numbers of adjectives marked by the subject to correctly indicate his/her current state of mood was counted (Eigenschaftswörterliste EWL-N; Janke and Debus, 1978).

2.3. Data reduction and analysis

Statistical analysis was based on analyses of covariance (ANCOVA) with two group factors representing the treatment condition and subject's sex. Values of the baseline sessions served as covariates. In case ANCOVA yielded a significant interaction of the factors treatment and sex, data were also analysed for male and female subgroups. A p-value < 0.05 was considered significant.

3. Results

3.1. Plasma hormones and glucose

Average plasma concentrations of insulin (Insulin vs. Placebo: 10.84 ± 1.31 vs. 9.83 ± 1.36 μU/ml; $F(1, 33) = 0.28$, non-significant, ns) and glucose (Fig. 1b) over the 8 weeks treatment period (Insulin vs. Placebo: 84.26 ± 0.8 vs. 86.12 ± 0.78 mg/dl; $F(1, 31) = 2.76$, ns) did not differ between groups. The same accounts for concentrations of epinephrine (Insulin vs. Placebo: 7.47 ± 0.71 vs. 6.66 ± 0.66 μg/l; $F(1, 29) = 0.67$, ns) and norepinephrine (Insulin vs. Placebo: 18.91 ± 2.91 vs. 20.56 ± 2.75 μg/l; $F(1, 27) = 0.76$, ns). Weekly measures of cortisol during Insulin treatment were constantly below concentrations of the Placebo group. After 8 weeks of treatment the decrease in serum cortisol (Insulin vs. Placebo: 14.03 ± 0.92

Table 1 Memory for words after acute and subchronic intranasal insulin administration

	Category	Insulin Mean ± SEM	Placebo Mean ± SEM	*P*-value
Baseline Period (Session 1)				
Immediate recall	Neutral	4.14 ± 0.29	4.34 ± 0.29	ns
	Emotion	4.11 ± 0.30	4.31 ± 0.30	ns
	Food	3.25 ± 0.35	3.17 ± 0.35	ns
	All words	11.60 ± 0.65	11.67 ± 0.65	ns
Delayed recall	Neutral	2.87 ± 0.47	2.66 ± 0.47	ns
	Emotion	3.04 ± 0.45	3.23 ± 0.45	ns
	Food	1.78 ± 0.34	2.01 ± 0.34	ns
	All words	7.68 ± 1.06	7.89 ± 1.06	ns
Acute Treatment (Session 2)				
Immediate recall	Neutral	4.14 ± 0.32	3.76 ± 0.32	ns
	Emotion	4.13 ± 0.27	4.18 ± 0.27	ns
	Food	3.84 ± 0.40	4.27 ± 0.40	ns
	All words	12.13 ± 0.68	12.19 ± 0.68	ns
Delayed recall	Neutral	1.68 ± 0.35	1.46 ± 0.34	ns
	Emotion	1.36 ± 0.36	1.50 ± 0.35	ns
	Food	1.40 ± 0.38	2.04 ± 0.37	ns
	All words	4.41 ± 0.95	5.03 ± 0.92	ns
Subchronic Treatment (Session 3)				
Immediate recall	Neutral	4.50 ± 0.47	4.14 ± 0.45	ns
	Emotion	4.72 ± 0.39	4.83 ± 0.42	ns
	Food	4.45 ± 0.44	4.65 ± 0.41	ns
	All words	13.82 ± 0.85	13.48 ± 0.81	ns
Delayed recall	Neutral	2.20 ± 0.37	0.86 ± 0.36	0.02
	Emotion	2.29 ± 0.45	0.94 ± 0.44	0.05
	Food	1.75 ± 0.42	1.08 ± 0.41	ns
	All words	6.20 ± 1.03	2.92 ± 1.00	0.04

Upper panel: Baseline period - Both groups (Insulin and Placebo) were treated with placebo. Recall of a word-list containing neutral, emotional and food-related words was tested immediately (3 min after presentation; Session 1) after the first intranasal administration of placebo (60 min before testing), and one week later (delayed recall) with the placebo treatment continued up to session 2, exclusively. Middle panel: Acute treatment – Presentation of word lists and immediate recall testing (Session 2) took place after the first intranasal administration of insulin (40 IU) and placebo, 60 min before testing. Delayed recall was tested 1 week later, with daily treatments of insulin (4×40 IU/d) and placebo continuing during the one week retention interval. Note that delayed recall testing did not grasp acute effects of insulin but those of a one-week treatment. Bottom: Subchronic treatment -Word list presentation and immediate recall (Session 3) were tested after a 7-week intranasal treatment with insulin (4×40 IU/d) and placebo. Delayed recall was tested one week later with the treatments continued throughout this week. Data are means ± SEM; values are baseline-adjusted as derived from ANCOVA. Right column indicates significant differences ($p < 0.05$) between the effects of treatments; non-significant (ns; $p > 0.1$).

Table 2 Rated mood after acute and subchronic intranasal insulin and placebo

	Insulin Means ± SEM	Placebo Means ± SEM	P-value
Acute treatment			
Activation	7.67 ± 0.99	6.1 ± 0.96	ns
Concentration	3.52 ± 0.39	3.51 ± 0.38	ns
Deactivation	4.81 ± 1.01	5.86 ± 0.98	ns
Fatigue	2.23 ± 0.47	2.52 ± 0.46	ns
Benumbed	0.98 ± 0.25	1.02 ± 0.24	ns
Extroversion	5.21 ± 0.50	4.17 ± 0.48	ns
Introversion	1.35 ± 0.51	1.62 ± 0.50	ns
Self-confidence	4.56 ± 0.51	3.05 ± 0.49	0.04
Well-being	9.30 ± 0.92	6.03 ± 0.89	0.02
Arousal	1.63 ± 0.35	1.88 ± 0.34	ns
Sensitiveness	0.88 ± 0.15	0.75 ± 0.15	ns
Anger	−0.02 ± 0.20	0.76 ± 0.19	0.01
Anxiety	0.49 ± 0.12	0.43 ± 0.12	ns
Depression	1.14 ± 0.53	2.08 ± 0.51	ns
Dreaminess	3.24 ± 0.45	2.98 ± 0.43	ns
Subchronic treatment			
Activation	7.87 ± 1.23	6.28 ± 1.17	ns
Concentration	3.78 ± 0.41	3.36 ± 0.39	ns
Deactivation	3.95 ± 1.15	5.36 ± 1.08	ns
Fatigue	2.58 ± 0.51	2.48 ± 0.48	ns
Benumbed	0.90 ± 0.28	1.40 ± 0.26	ns
Extroversion	5.53 ± 0.43	3.84 ± 0.41	0.01
Introversion	0.76 ± 0.41	1.59 ± 0.39	ns
Self-confidence	4.75 ± 0.50	3.17 ± 0.47	0.03
Well-being	9.56 ± 1.03	6.61 ± 0.98	0.05
Arousal	2.01 ± 0.49	1.93 ± 0.47	ns
Sensitiveness	0.94 ± 0.32	1.32 ± 0.31	ns
Anger	0.29 ± 0.30	0.74 ± 0.29	ns
Anxiety	0.43 ± 0.23	0.93 ± 0.21	ns
Depression	1.01 ± 0.64	3.15 ± 0.61	0.02
Dreaminess	3.51 ± 0.57	2.86 ± 0.54	ns

Scales scores for both treatment conditions are shown (Insulin and Placebo). Values are baseline-adjusted mean scores (± SEM). Right column indicates significant ($p < 0.05$) and non-significant (ns; $p > 0.1$) differences between the effects of treatments.

vs. 18.15 ± 1.76 μg/dl; $F(1, 36) = 5.3$, $p < 0.05$) reached significance (Fig. 1c).

3.2. Word list learning, Wordstem priming and Stroop test

Results regarding the declarative word list learning task are summarized in Table 1. Insulin administered acutely before learning did not influence immediate or delayed recall of words. Prolonged intranasal intake of insulin over 8 weeks significantly improved the delayed recall of words. The insulin-treated subjects recalled significantly more emotional and neutral words and this effect was also observed for the total word list. Recall of food-related words per se was not enhanced (Table 1). There was no significant interaction between the effects of treatment and word category ($F(1, 69) = 1.16$, ns). Also, prolonged treatment with insulin did not affect immediate recall of words ($p > 0.24$ for all comparisons). Performance on the wordstem priming task did not differ between conditions, neither after acute administration (Insulin vs. Placebo: 4.98 ± 0.55 vs. 4.65 ± 0.55 words; $F(1, 35) = 0.18$, ns) nor after subchronic treatment (Insulin vs. Placebo: 4.25 ± 0.53 vs. 3.88 ± 0.50 words; $F(1, 33) = 0.25$, ns). Likewise, performance did not differ between conditions for the three 3 Stroop results, the reading test (acute, Insulin vs. Placebo: 102.02 ± 1.21 vs. 103.25 ± 1.15 correct answers; $F(1, 33) = 0.54$, ns; subchronic, Insulin vs. Placebo: 102.84 ± 1.71 vs. 104.83 ± 1.71 correct answers; $F(1, 33) = 0.67$, ns), the color naming test (acute, Insulin vs. Placebo: 88.99 ± 2.10 vs.

89.12 ± 1.98 correct answers; $F(1,33) = 0.00$, ns; subchronic, Insulin vs. Placebo: 86.73 ± 2.76 vs. 82.38 ± 2.76 correct answers; $F(1,33) = 1.22$, ns), and the interference test (acute, Insulin vs. Placebo: 66.72 ± 2.19 vs. 62.72 ± 2.07 correct answers; $F(1,33) = 1.41$, ns; subchronic, Insulin vs. Placebo: 59.67 ± 2.16 vs. 58.39 ± 2.16 correct answers; $F(1,33) = 0.17$, ns). Also, there were no sex-specific effects of the treatment ($p > 0.25$ for all respective treatment $\times$ sex interactions).

3.3. Mood and subjective data

Administration of insulin acutely raised feelings of well-being ($F(1,34) = 6.47$, $p < 0.02$) and self-confidence ($F(1,34) = 4.37$, $p < 0.04$), and decreased rated anger ($F(1,34) = 7.86$, $p < 0.01$) in comparison with placebo (Table 2). Scores for extroversion were acutely enhanced after insulin administration in the men (Insulin vs. Placebo: 6.09 ± 0.52 vs. 3.99 ± 0.52; $F(1,21) = 7.89$, $p < 0.01$) but not in the women (Insulin vs. Placebo: 3.32 ± 1.03 vs. 4.58 ± 0.94; $F(1,10) = 0.76$, ns; $F(1,32) = 3.94$, $p < 0.05$ for treatment $\times$ sex). After 7 weeks of treatment, well-being ($F(1,33) = 4.25$, $p < 0.05$) and self-confidence ($F(1,33) = 5.22$, $p < 0.03$) again reached higher values in the insulin than placebo group. Likewise, scores of extroversion were generally enhanced independent of gender ($F(1,33) = 7.62$, $p < 0.01$), and feelings of depression were decreased ($F(1,33) = 5.88$, $p < 0.02$) in the insulin group. Interviews conducted at the end of the treatment phase did not reveal any hints at side effects of the treatment. Four subjects of the insulin group and 5 subjects of the placebo group believed that they had been treated with an active agent.

4. Discussion

Intranasal intake of insulin enhanced long-term declarative memory in humans without causing systemic side effects like hypoglycaemia. The improvement of memory in the eighth week of treatment corroborates previous findings of improved memory function following acute intravenous administration of the peptide both in healthy subjects (Kern et al., 2001) and in patients with Alzheimer's disease (Craft et al., 1999). In addition, intranasal insulin positively affected mood in our subjects. The improving effect of subchronic intranasal insulin administration appeared to be specific for hippocampus dependent declarative memory. There were no signs of general changes in attention and arousal following insulin. Self reported activation and concentration did not differ between the insulin and placebo groups at any time. Reading of color names, naming of colors as well as the interference test of the Stroop test likewise did not indicate any influence of insulin on selective attention. Wordstem priming chosen to examine a non-declarative type of memory not depending on hippocampal function did not differ between treatment groups, neither acutely nor after subchronic treatment.

Regarding the enhanced delayed recall of words after subchronic insulin treatment, it might be argued that this reflects an influence on encoding of the words at learning or a direct influence on retrieval, rather than an effect on the proper consolidation of memory. However, this view seems unlikely in light of the fact that neither the acute nor the subchronic administration of insulin benefited the immediate recall of the word list. Although slight separate effects of insulin on these processes cannot be completely ruled out based on our study design, the pattern of changes thus speaks for a most prominent effect of subchronic insulin on the consolidation of declarative memory. It is also to be considered that the improvement in delayed recall testing in the insulin-treated group occurred on a background of a generally decreasing performance. The decrease in delayed recall performance not only reached significance ($p < 0.01$) between the second and third testing in the placebo group but was likewise present in both the insulin and placebo groups between the first and second testing (Table 1). The most plausible, yet post hoc explanation of this phenomenon refers to proactive interferences. In fact, inspection of data indicated that on the last delayed recall testing, performance was considerably diminished by intrusions, i.e., falsely recalled words from previous word lists. However, this interference effect was closely comparable for both groups ($p > 0.41$). Accordingly it cannot explain the improvement in delayed recall seen at week 8 of treatment selectively in the insulin-treated subjects. It is also to note in this context, that the delay between learning and recall testing here was one week which is substantially longer than the delay of 20–30 min more frequently used for experimental memory testing. The formation of memories over such a long interval may involve processes of consolidation additional to those underlying testing after shorter intervals. Also, memory consolidation over a longer interval is probably more prone to interfering disturbances. Nevertheless, an one-week interval can be considered a rather valid indicator of long-term memory (Dudai, 2004).

Our subjects in the insulin group also expressed enhanced mood. Acute intranasal intake of insulin enhanced the feelings of well-being and self-confidence, which is in accordance with previous results (Kern et al., 1999). With subchronic treatment of intranasal insulin, feelings of well-being and self-confidence remained enhanced, and subjects felt more extroverted and less depressed. Considering its early onset and differently improved dynamics the generally enhanced mood after insulin appears to represent a separate temporal psychological influence of the peptide mediated by mechanisms different from those involved in improving declarative memory. Recall of positive emotional words may have slightly benefited from the improved mood (Van Honk et al., 2003). However, the positive emotional words represented a too small number of items to explain the overall enhanced memory for the total list of words. The increasing effect of subchronic administration of insulin on extroversion being restricted to the men might be related to a modulatory role of gender on the brain action of insulin, as has been likewise revealed for effects of insulin on body weight (Clegg et al., 2003). These findings point to a relevant sexual dimorphism in the sensitivity to the effects of insulin on brain functions. However, it is presently not clear how this gender difference is mediated.

The mechanisms underlying the improved declarative memory following subchronic insulin treatment are not known. Brain insulin receptors are expressed at a high density in the hippocampus (Unger et al., 1991) known to be essential for declarative memory formation (Squire, 1992; Eichenbaum, 2001). The slow onset of the action of insulin with the absence of any acute changes in memory function speaks for an effect involving gradual plastic neuronal changes. In diabetic rat models, a lack of insulin triggered a retraction of dendrites and reduced NMDA transmission of hippocampal neurons resulting in an atrophy of the hippocampus and diminished memory performance (Magarinos and McEwen, 2000; Gardoni et al., 2002). Moreover, insulin also influences glial cells known to enhance synaptic plasticity via the release of neurotrophic factors (Wozniak et al., 1993; Fields and Stevens-Graham, 2002). Thus, prolonged increases in central nervous insulin may not only protect but also support the development of neural connectivity in hippocampal brain regions resulting in improved declarative memory.

The enhanced consolidation of words after subchronic insulin treatment could also be related to the decrease in plasma cortisol concentrations seen at this session. Glucocorticoids have been shown to impair declarative memory formation by binding to hippocampal glucocorticoid receptors, thereby inhibiting synaptic long-term potentiation and decreasing hippocampal glutamate turn-over (Sapolsky, 1993; Plihal and Born, 1999b). Also, long-term increases in corticosteroids are associated with reduced branching of hippocampal dendrites (Bisagno et al., 2000; Sandi et al., 2001) and atrophy of the hippocampus (Lupien et al., 1998). Thus, the small decrease in cortisol concentrations seen in the insulin-treated group after subchronic treatment with insulin may have added to the improvement of hippocampal-dependent memory of the words. However, additional analysis did not yield significant correlations between plasma concentrations after 8 weeks and delayed recall of words so that this interpretation remains tentative. The decrease in cortisol could also be valued as a further hint at improving effects of insulin on hippocampal functioning. The hippocampus is considered to exert an inhibiting control over the hypothalamo-pituitary-adrenal system via direct and indirect projections to the paraventricular nucleus and the ventromedial region of the hypothalamus (Jacobson and Sapolsky, 1991; Born and Fehm, 1998). This inhibitory influence becomes effective in particular under basal conditions and reflects input of the integrative processing of corticosteroid feed back signals via mineralocorticosteroid receptor expressing neurons in the hippocampus (de Kloet et al., 1998). The decrease in cortisol could thus point to an enhanced corticosteroid feedback processing in the hippocampus following intranasal insulin administration.

In summary our data indicate that prolonged intranasal intake of insulin improves both consolidation of words and general mood. These beneficial findings suggest intranasal administration of insulin as a potential treatment in patients showing memory deficits in conjunction with a lack of insulin, such as in Alzheimer's disease (Craft et al., 1998).

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