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Manipulating neuropeptidergic pathways in humans: a novel approach to neuropharmacology?

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Abstract

Given the tremendous number of neuropeptides, which are synthesized in the central nervous system, the brain can be viewed as one of the most prominent endocrine organs. Elucidation of the functions of these peptides is hampered by the facts that after intravenous administration access to brain receptors is prevented or impaired by the blood–brain barrier. Here, we provide evidence that intranasal administration can be a way to circumvent the blood–brain barrier. Selected experiments will be reported indicating that peptides after intranasal administration in humans can specifically alter a great variety of brain functions. For vasopressin, we demonstrated improving effects of long-term intranasal treatment on sleep in elderly people. Insulin showed improving effects of short-term memory functions. For adrenocorticotropin/melanocyte stimulating hormone, ACTH/MSH-(4–10), a twofold action was isolated: The melanocortin fragment diminished selective attention and, with subchronic administration, reduced body fat. These results could provide the basis for developing a new, specific, and "soft" neuropharmacology.

Keywords: Neuropeptide; Intranasal administration; Vasopressin; Insulin; Melanocortin; Cognition

1. Introduction

Among the first neuropeptides discovered were angiotensin II and vasopressin. The progress in molecular biology was accompanied by a bewildering increase in the number of established neuropeptides and brought into life a "never ending stream of neuropeptides". For many of these peptides, data concerning their molecular biology, biochemistry and neuroanatomical distribution were accumulated in fascinating detail. However, for most of them, a physiological or neurobehavioral role remains to be specified. This is apparently the consequence of their location beyond the blood–brain barrier, which protects the neuropeptidergic system within the brain from peripheral interferences and also from scientific scrutiny.

Heroic efforts were necessary to overcome these difficulties when De Wied and van Ree set out many years ago to study the effects of peripheral administration of adrenocorticotropin (ACTH) and vasopressin on higher brain functions in experimental animals. They could demonstrate that processes related to learning, memory, and attention are indeed influenced by these peptides. In particular, they showed also that the 4–10-fragment of ACTH is sufficient for eliciting the same neurobehavioral effects as the whole ACTH molecule. Notably, the 4–10-fragment of ACTH represents a core sequence of all melanocortins, and ACTH-(4–10) is identical with melanocyte stimulating hormone, MSH-(4–10). We are proud to be among the first who attempted to transport those fundamental findings by De Wied and van Ree into human physiology, and could eventually confirm in human subjects many of their observations in rats.

There is no doubt that peptides administered systemically at large doses can overcome the obstacles brought about by the blood–brain barrier. However, for many peptides with receptors both on the systemic as well as on the brain side of the blood–brain barrier, this approach will be limited by side effects due to peripheral actions. Therefore, a way around the blood–brain barrier would represent a major step forward in the dissociation of peptidergic effects on brain function. There is evidence that after intranasal administration peptides have direct access to the cerebrospinal fluid compartment and, hence, to their re-

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spective brain receptors. This evidence is provided in the following. Consecutively, results exploiting this approach for the investigation of central nervous effects in humans are presented for three classical peptides studied in psychoneuroendocrinology: vasopressin, insulin, and ACTH/MSH-(4–10).

2. Methods: intranasal administration of neuropeptides

Since the olfactory nerves are surrounded by a space that contains liquor and is continuous with the subarachnoidal space, after intranasal administration, peptides may diffuse directly into the cerebrospinal compartment through the olfactory epithelium. This view is substantiated by findings in rats and primates showing that larger tracer molecules, such as horseradish peroxidase, pass freely through intercellular junctions of the olfactory epithelium to reach extracellularly the fiber layer of the olfactory bulb within minutes (Balin et al., 1986). Peroxidase in those studies diffused out of the olfactory fiber layer over time, presumably as a consequence of bulk flow of cerebrospinal fluid. Likewise, several viruses and drugs like cephalexin have been demonstrated to enter the brain via a passage through the olfactory epithelium (Barnett and Perlman, 1993).

Convergent functional evidence that effects of vasopressin on human cognitive function after intranasal administration derive from a direct access of the peptide to the brain has been provided by Pietrowsky et al. (1996). That study in healthy men compared the effects of arginine-vasopressin on event-related brain potential responses to tones, after the intranasal and the intravenous route of administration. The doses and the rate of the intravenous infusion were chosen so that increases in plasma concentrations of vasopressin were equal to or substantially higher than those observed following the intranasal route of administration of 20 IU vasopressin. While intranasal vasopressin induced a distinct increase in amplitude of the P3 component of the event-related brain potential response to tones, intravenous infusion of vasopressin at all plasma concentrations remained completely ineffective (Fig. 1).

Insulin in circulating blood has rapid access to the brain via the circumventricular organs lacking a blood–brain barrier, and also via a receptor mediated transport system located in endothelial cells of brain microvessels (Schwartz et al., 1992). In several species including man, parallel changes in plasma and brain interstitial and cerebrospinal fluid insulin concentrations are well documented (Schwartz et al., 1990). In the brain, insulin receptors are widely distributed with highest densities in the olfactory bulb, hypothalamus and hippocampus and related limbic brain structures (Unger et al., 1991).

Although multiple effects of insulin on single neurons and isolated brain structures have been demonstrated (Schwartz et al., 1992; Unger et al., 1991), the consequence of insulin effects for brain function in humans remains unclear. This lack of human data mainly derives from the fact that direct central nervous actions of insulin after systemic administration are difficult to dissociate from effects due to the concomitant decrease of blood glucose concentration. Therefore, most evidence regarding influences of insulin on human brain function so far was derived from rather complex methodical approaches, such as the euglycemic and hypoglycemic clamp techniques.

A promising alternative strategy for the assessment of brain effects of insulin derived from the investigation of changes after intranasal administration of insulin. Intranasally administered insulin without absorption enhancers is known to be hardly absorbed into the blood (Illum and Davis, 1992). Thus, intranasal insulin may gain access to the brain without involving confounding effects

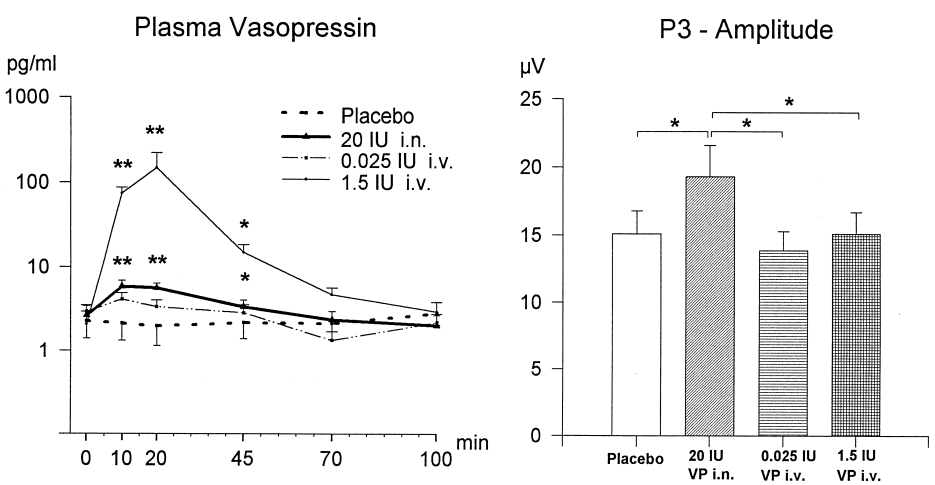

Fig. 1. Left: Plasma vasopressin (VP) levels following 20 IU arginine vasopressin (AVP) intranasal (i.n.), 0.025 IU AVP intravenous (i.v.), 1.5 IU AVP i.v., and placebo at 0, 10, 20, 30, 45, 70, and 100 min following administration. Right: Peak amplitude of the P3 component of the event-related brain potential following placebo, and i.n. as well as i.v. AVP administration.

due to systemic actions of the hormone. In fact, pilot experiments in our laboratory in eight healthy humans examining cerebrospinal concentrations of insulin indicated a distinct accumulation of the peptide in cerebrospinal fluid following intranasal administration. In all of these subjects, the single intranasal administration of 40 IU insulin induced a sharp rise in cerebrospinal fluid insulin concentrations 10–20 min later, which was not accompanied by a corresponding rise in serum insulin levels (Kern et al., submitted).

Recently, we assessed systematically to what extent ACTH/MSH-(4–10) enters the human brain compartment after intranasal administration (Fehm et al., submitted). In young healthy subjects, cerebrospinal fluid samples were collected between 15 min before and 75 min after intranasal administration of the peptide fragment at a dose of 10 mg. The peptide was administered as repeated intranasal puffs to each nostril within 10 min. Cerebrospinal fluid was sampled via an intraspinal catheter while the subject sat in an upright position. ACTH/MSH-(4–10) in cerebrospinal fluid and serum was determined by radioimmunoassay described in detail elsewhere (Bickel et al., 1988).

As exemplified in Fig. 2 by the curves from three individuals, concentrations of ACTH/MSH-(4–10) in cerebrospinal fluid showed distinct elevations within about 70 min after intranasal administration of the peptide. Concurrent changes in serum concentrations were distinctly smaller. Together, this data indicate access of ACTH/MSH-(4–10) to the brain via the intranasal route of administration, which does not appear to rely on prior resorption of the substance into the blood stream.

3. Results: neurocognitive effects of neuropeptides in humans

Here, selected experiments will be reported indicating that peptides after intranasal administration in humans can specifically alter a great variety of brain functions. These alterations may eventually turn out to be also of clinical importance for the treatment of certain brain diseases. For vasopressin, we demonstrated distinctly improving effects of a long-term intranasal treatment with vasopressin on sleep in elderly people. Insulin showed improving effects on short-term memory function. For ACTH/MSH-(4–10), a twofold action was isolated: The melanocortin fragment diminished selective attention function and, with subchronic administration, reduced body fat.

3.1. Vasopressin and sleep

Numerous animal studies demonstrated central nervous effects of vasopressin after systemic and intracerebroventricular administration besides its regulatory action on fluid balance and circulation. Vasopressin seemed to play a role for memory and learning in animals (Van Wimersma Greidanus and Van Ree, 1990). Also, in healthy humans, significant effects on signs of attention and learning were reported following administration of vasopressin (Born et al., 1998). However, attempts to improve specifically memory performance in various pathological conditions like dementia and post-traumatic disorders revealed rather inconsistent results (De Wied and van Ree, 1989). Likewise, experiments failed, which aimed to compensate for cognitive impairments in elderly by the administration of vasopressin (Dodt et al., 1994; Nebes et al., 1984).

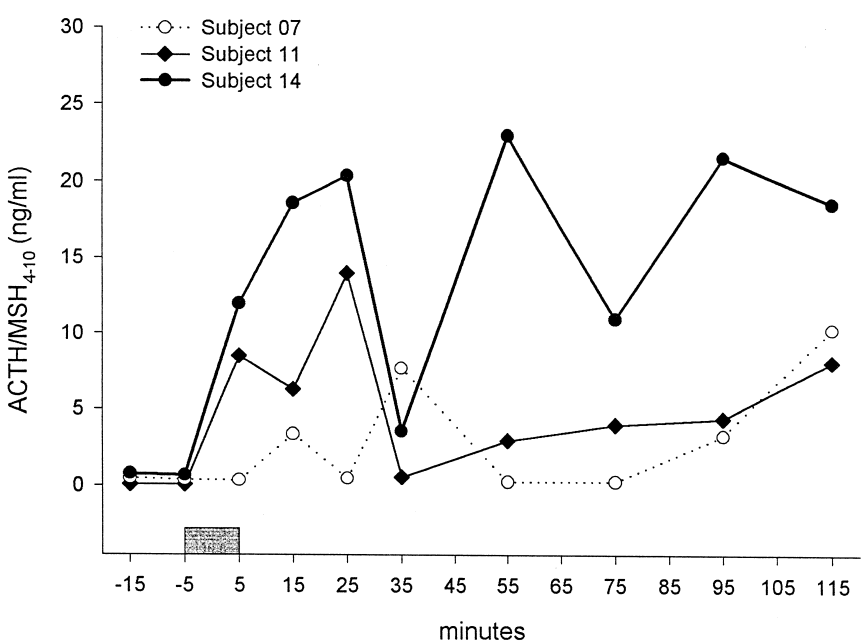

Fig. 2. Time course of cerebrospinal fluid concentrations of ACTH/MSH-(4–10) after intranasal administration of 10 mg of the peptide in three individuals.

Despite the well-known association of vasopressinergic brain structures with brain regions involved in the regulation of the sleep–wake cycle, only few studies were undertaken to investigate the effects of vasopressin on sleep. Experiments with Brattleboro rats suffering from a hereditary lack of vasopressin, showed inconsistent results. A reduced amplitude of slow wave sleep (SWS) and paradoxical sleep (PS) in these rats has been reported (Brown and Nunez, 1989). However, intracerebroventricular administration of vasopressin did not change PS of the animals, but increased hippocampal theta rhythm (Urban and DeWied, 1978). Moreover, the sleep deficit in Brattleboro rats has been attributed to the need of drinking, rather than to the absence of vasopressin, because sleep was improved in these animals after infusion of daily water intake (Danguir, 1983). Compared with controls, normal rats after intracerebroventricular administration of vasopressin spent more time awake and displayed an increased amplitude of the circadian sleep–wake rhythm (Arnauld et al., 1989; Kruisbrink et al., 1987). In particular, these latter results appear to be consistent with the widely held view that acute administration of the peptide primarily acts to increase general central nervous arousal.

Three studies examined acute effects after systemic application of vasopressin and vasopressin-analoga on sleep in young healthy human subjects. Timsit-Berthier et al. (1982) found increased sleep stage 2 after intranasal administration of 14 IU lysine-vasopressin. Using continuous intravenous infusion of vasopressin at doses of 0.33, 0.66 and 0.99 IU/h, we also observed increased sleep stage 2 accompanied by reduced rapid eye movement (REM) sleep and increased wake time on the vasopressin conditions (Born et al., 1992). Snel et al. (1987) investigated self-rated sleep quality and sleepiness, and did not reveal any effect of an intranasal arginine-vasopressin analog. Together, the results suggest a flattening of sleep following acute administration of vasopressin in healthy humans thus, matching with respective data in animals. The effect could be attributed to a primary enhancing action of vasopressin on central nervous arousal.

However, in comparison with the acute effects in young healthy humans, effects of vasopressin may be different in conditions where endogenous vasopressin is lacking, and also with prolonged administration of the substance. This in mind, we launched a pilot study in two aged humans examining a great variety of neurocognitive functions and also sleep during a 3-month period of daily treatment (40 IU) with vasopressin. Vasopressin content of the aged brain is known to be diminished in a number of structures including also the nucleus suprachiasmaticus of the hypothalamus, a region essential for the occurrence of normal sleep–wake oscillations (Swaab, 1995). The prolonged treatment with vasopressin did not change behavior and event-related brain potential indicators of cognitive function in the elderly. However, the peptide dramatically increased the time these subjects spent in SWS, by more than 100% with, this increase emerging 6 weeks after treatment had started (Perras et al., 1996). This was unexpected, since the SWS promoting effect developing with prolonged treatment with vasopressin was opposite to the observation after an acute single administration of the peptide, where increased portions of light sleep and awake time occurred after vasopressin administration.

To further substantiate this finding, a double blinded, placebo controlled study was undertaken in a larger sample of 26 healthy elderly (14 women, 12 men). The participants of this study were all over 70 years, took no medication and did not complain about poor sleep. However, sleep recordings revealed a fragmented sleep pattern in these subjects, which is typical for elderly persons, and is dominated by sleep stages 1 and 2 with little amounts of SWS and REM sleep (Prinz et al., 1990). Each subject initially spent three nights at the sleep laboratory and received intranasally placebo (saline solution). After the third night, subjects were randomly assigned to two treatment groups. One group ($n = 13$) continued to take placebo, while the other group received a total dose of 40 IU of vasopressin/day (20 IU in the morning and 20 IU in the evening). Treatment was continued for 3 month and followed by another three nights at the sleep laboratory for polysomnographical recordings. Results of the pilot study were confirmed (Perras et al., 1999). Subjects after subchronic treatment with vasopressin slept on average 45 min longer than following placebo, and spent about 20 min more in SWS and 10 min more in REM sleep (Fig. 3, Table 1). No cardiovascular side effects or effects on fluid retention were observed.

Together, the experiments so far demonstrate vasopressin effects on human sleep, which appear to depend on whether effects of acute or prolonged intranasal administration are examined. Acutely, an arousing influence of vasopressin is dominant, which might involve a direct action of the peptide on cholinergic, reticular brainstem regions responsible for the regulation of general central nervous arousal level in the course of the sleep–wake cycle. However, the somnogenic effects of vasopressin after long-term administration require other explanations, since they developed not until a couple of weeks after treatment with vasopressin had started. Related studies in animals and humans point to a possible involvement of central nervous mineralocorticoid receptors in the mediation of this effect. Mineralocorticoid receptors in the brain are most densely expressed in the hippocampus, and in rats, prolonged treatment with vasopressin increased capacity of hippocampal mineralocorticoid receptors (Veldhuis and De Kloet, 1982). In addition, in humans blocking of mineralocorticoid receptors after administration of canrenoate distinctly reduced the time in SWS whereas stimulation of these receptors after moderate doses of cortisol induced a significant enhancement of SWS (Born et al., 1991, Friess et al., 1994). Together, these observations lend themselves to speculate that the profound increase in

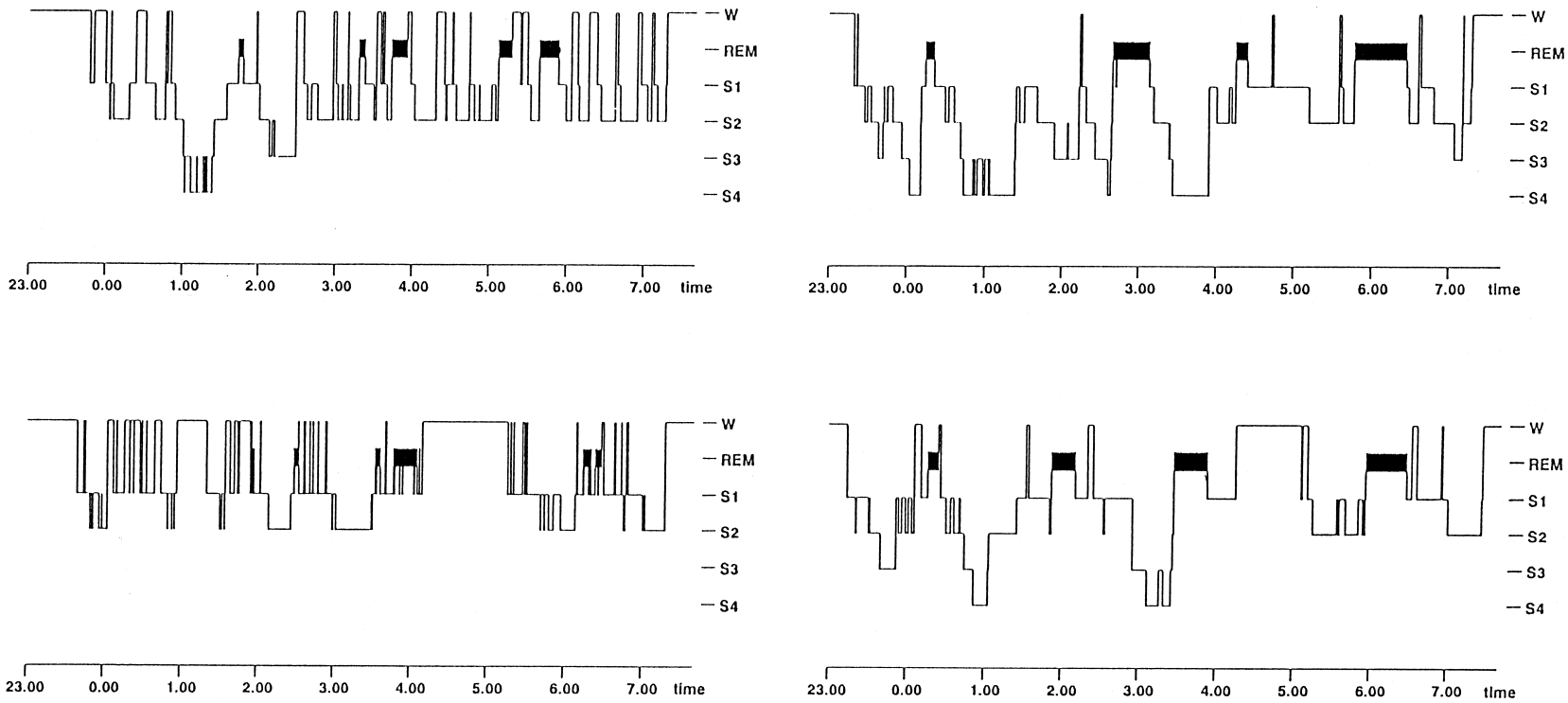

Fig. 3. Sleep profiles from two selected subjects (aged 70 and 76 years) before (left panel) and after (right panel) vasopressin treatment. Note increased sleep time, SWS and REM sleep in both subjects after prolonged treatment with the peptide. The number of awakenings also seemed to be reduced after vasopressin. Both subjects turned off the lights at 11 p.m. W, awake time.

Table 1
Time spent in the different sleep stages was determined for the total sleep period time (SPT) and also separately for the first and second half of SPT. Right columns indicate F and p values for pairwise comparisons ($df = 1,23$). Level of significance, $p < 0.05$. Sleep onset latency: time between lights off and the onset of the first period of sleep stage 1 (S1) followed by sleep stage 2 (S2); sleep period time: time between sleep onset and final awakening; total sleep time: time spent asleep between sleep onset and final awakening; sleep efficiency: SPT minus time awake relative to total SPT (in %); WASO, time awake after sleep onset; SWS, slow wave sleep (S3 + S4); REM, rapid eye movement sleep

	Placebo ($n = 13$)		Vasopressin ($n = 13$)		F value	p value
	mean	S.E.M.	mean	S.E.M.		
Sleep onset latency (min)	26.8	4.9	22.0	2.3	0.64	0.43
Sleep period time (min)	404.0	18.2	449.2	12.2	12.34	0.002
Total sleep time (min)						
Sleep efficacy (%)	86.9	2.1	86.9	1.9	0.00	0.99
Time of sleep stages (min)						
WASO	55.1	9.5	58.9	9.2	0.16	0.69
S1	81.2	12.9	75.1	13.4	0.86	0.36
S2	170.4	12.3	187.2	13.0	3.42	0.07
SWS (S3 + S4)	36.5	6.0	58.0	9.4	5.52	0.025
REM	60.8	5.7	70.0	6.3	1.83	0.19
Time of sleep stages (%)						
WASO	13.5	2.1	13.1	1.9	0.00	0.99
S1	20.1	2.1	16.7	3.0	3.59	0.07
S2	42.2	3.1	41.7	2.8	0.09	0.77
SWS (S3 + S4)	9.1	1.7	13.0	2.0	3.99	0.06
REM	15.1	1.1	15.5	1.4	0.08	0.79
Sleep stages 1st half (min)						
WASO	27.1	6.0	26.4	6.5	0.00	0.95
S1	44.1	7.0	38.2	8.3	0.24	0.60
S2	77.8	7.5	94.8	6.6	4.20	0.055
SWS (S3 + S4)	24.2	5.5	37.0	5.9	4.87	0.038
REM	30.1	3.3	28.2	3.8	0.01	0.92
Sleep stages 2nd half (min)						
WASO	27.4	4.3	33.0	5.2	1.14	0.30
S1	43.9	6.3	37.4	6.1	0.51	0.48
S2	86.4	5.7	91.3	6.5	0.23	0.64
SWS (S3 + S4)	12.1	1.9	20.9	4.7	3.38	0.079
REM	31.7	3.6	42.0	3.0	7.98	0.01

sleep and SWS seen in aged persons after subchronic intranasal vasopressin administration could be a consequence of activated expression of hippocampal mineralocorticoid receptors. Also, vasopressin could compensate for the age-related reduction of vasopressin in the nucleus suprachiasmaticus, thereby improving vasopressin dependent output of the circadian pacemaker. In light of this explanation also enhancing effects of vasopressin on daytime activity would be expected, which have not been tested in humans so far.

3.2. Insulin and cognitive functions

Insulin receptors are widely distributed in the brain with, particularly high concentrations in the olfactory bulb and hippocampus. However, receptors are present also in the neocortex. Activation of these receptors may mediate diverse effects on cognitive function. To outline main features of the profile of neurobehavioral effects of insulin in humans, we performed a series of studies mainly relying on event-related brain potential indicators of cognitive processing. Earlier components of the event-related brain potential response such as the N1 and P2 generated within 150 ms after onset of a stimulus, reflect earlier steps of stimulus processing at preattentive stage. In contrast, late event-related brain potential components emerging after 150 ms post-stimulus onset, such as the P3 component and 'slow wave' related potential activity, are commonly considered signs of conscious stimulus processing at an attentive stage, occurring once the stimulus has entered working or short-term memory.

Considering the direct access of peptides to brain structures after intranasal administration, in a recent study for the first time we evaluated the effects of insulin after the intranasal route of administration (Kern et al., 1999, 2000). In healthy subjects, a dose of 20 IU was given every 15 min over 60 min, and event-related brain potential to auditory stimulus trains were recorded in the context of an

attention task. No measurable and biologically relevant amount of insulin entered the blood, since concentrations of serum insulin and blood glucose were similar during conditions of intranasal insulin versus placebo administration. This definitely excludes that changes observed for cognitive function parameters after intranasal insulin were mediated via absorption of the substance into the bloodstream (Fig. 4).

The primary changes in event-related brain potential responses after intranasal administration of insulin were a decrease in amplitude of the early N1 component and an increased negative potential between 300 and 700 ms post-stimulus, which is a latency range where P3 and slow wave overlap. The negative potential shift after intranasal insulin administration focussed over frontal cortical regions, which precludes an effect on the classical P3 component commonly dominating over posterior, i.e., parietal cortical areas. Rather, the shift reflects a direct enhancing effects of insulin on a frontal negative 'slow wave'. The same increase in negative slow wave activity in the event-related brain potential over frontal cortical regions was observed in a parallel study examining effects of insulin after intravenous infusion (Kern et al., submitted). The study compared effects of 6-h intravenous insulin infusions at two doses (15.0 versus 1.5 mU/kg min) while blood glucose was kept at normal levels (euglycemic clamp conditions), and a striking negative slow wave emerged with administration of the higher dose of insulin. Further studies showed a similar enhancement in the frontocortical negative slow wave of visually evoked event-related brain potential responses following insulin-induced hypoglycemia in healthy subject (Smid et al., 1997) and in patients with type 2 diabetes (Lobmann et al., 1998).

At a psychological level, the frontal negative 'slow wave' has been related to an increased allocation of processing resources (Rockstroh et al., 1989). Thus, slow wave negativity points to an increased recruitment of attentional capacities upon presentation of a stimulus, thereby eventually improving encoding of the stimuli into working memory. This view is consonant with concurrent changes in behavioral measures observed after insulin administration. Thus, the increase in frontal negative slow wave observed during intravenous insulin infusion under euglycemic clamp conditions was paralleled by signs of improved selective attention (as assessed by the Stroop interference test) and enhanced short term memory (Kern et al., submitted).

The improving effect of insulin on short-term memory points to an action on hippocampal function. The hippocampus in conjunction with surrounding medial temporal cortex is known to play a pivotal role in the conscious explicit acquisition and recall of so-called declarative memories, which include memories for words and stories, but also the memory for dates (e.g., birthdays) and spatial locations (e.g., of a restaurant). The hippocampus is among the brain regions with the greatest number of insulin binding sites. Raising plasma insulin levels during euglycemia in rats results in increased insulin binding in the hippocampal CA1 region (Marfaing et al., 1990). Insulin binding in the hippocampus is associated with an increase in immunocytochemically detectable phosphotyrosine and insulin receptor substrate-1, the putative cellular intermedi-

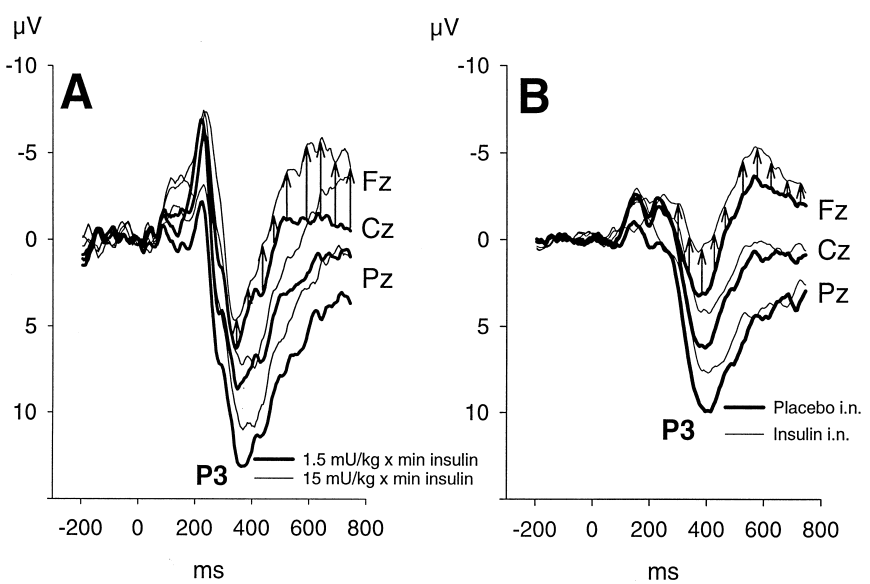

Fig. 4. Grand average evoked potential responses (A) after 360 min of insulin infusion at a rate of 15 mU/kg min (thin lines) or 1.5 mU/kg min (thick lines), (B) after 60 min of intranasal treatment with 20 IU insulin every 15 min (thin lines) or placebo (thick lines), recorded at frontal (Fz), central (Cz) and parietal (Pz) electrode positions. To isolate the "pure" P3 component, the average evoked potential curve for standard tones was subtracted from the AEP curve to target tones. Arrows indicate the broad negative potential shift over the frontal recording site observed in both experiments.

ates of the insulin action (Baskin et al., 1994, 1993). Intracerebroventricular administration of insulin has also been shown to increase hippocampal glucose utilization (Henneberg and Hoyer, 1994), whereas lesioning of brain insulin receptors with streptozotocin disrupts hippocampal glucose utilization (Plaschke and Hoyer, 1993).

It is also possible that the improvement in short-term memory function following insulin was a consequence of enhanced selective attention facilitating the encoding of relevant information. This view would account for the improved performance on the Stroop interference task following administration of insulin. The Stroop task requires the subject to name as fast and as accurately as possible the colour in which words are printed while processing of the word meaning (representing the distracting influence) has to be suppressed. Studies in brain lesioned patients (Lezak, 1993) and brain imaging studies using positron emission tomography (Taylor et al., 1997) indicated that this type of selective attention performance relies essentially on the integrity of frontal cortical regions. Thus, both improved performance on the Stroop interference task as well as the finding of a distinctly increased frontal slow wave in the event-related brain potential speak for a primary effect of insulin on attentional mechanisms residing in the frontal cortex. Overall data support a regulatory influence of insulin on a hippocampo-frontocortical circuitry, which primarily serves to facilitate the allocation of attentional resources to stimuli to be encoded within working memory (Okita et al., 1985; Näätänen, 1982).

This action of insulin appears to be of potential clinical relevance in light of recent reports that in patients with Alzheimer's dementia concentrations of insulin in cerebrospinal fluid are decreased (Craft et al., 1998). Moreover, the decrease was found to be positively correlated with the severity of dementia. Experimental elevation of insulin levels in these patients under euglycemic conditions improved immediate and delayed recall of stories (Craft et al., 1996). These results indicate that the lack of insulin in the brain of Alzheimer patients belongs to the factors significantly contributing to the memory impairment in these patients.

In sum, stimulation of central nervous insulin receptors in humans appears to weaken information processing at early, pre-attentive stages, as indicated by reduced amplitudes and prolonged latencies of event-related brain potential components, such as the N1, following insulin administration. At later, attentive stages of stimulus processing insulin has an improving influence as reflected by the distinct increase in the frontal negative slow wave of the event-related brain potential, in conjunction with improved selective attention and short-term memory function and subjective feelings to think with greater ease. The pattern of changes in cognitive function after insulin probably reflects an action on hippocampo-frontocortical circuitry responsible for the encoding of relevant stimuli into working memory. During encoding, the hippocampus is known to mediate an inhibitory control over arousing structures at the midbrain level, i.e., the ascending reticular activating system (Vinogradova, 1999). This inhibition would explain the finding of decreased amplitudes and prolonged latencies of early event-related brain potential components after insulin administration, the generation of which involves activation of these midbrain structures. The concurrent inhibition of the ascending reticular activating structures during encoding within hippocampo-frontocortical circuitry presumably represents another prerequisite optimising the process of stimulus encoding into working memory.

3.3. ACTH / MSH-(4–10), selective attention and the regulation of body weight

The investigation of ACTH/MSH-related peptides revealed two very different types of influences for this group of peptides, exemplifying the diversity of neurobehavioral effects that can be exerted by an individual neuropeptide. Melanocortins appear to play an essential role in the regulation of selective attention and also of body weight. In humans, the behaviorally active fragment ACTH/MSH-(4–10) as well as analogs of this sequence such as ORG 2766 have been consistently found to weaken signs of selective attention (Born et al., 1986, 1987). The parameter most sensitive to this influence was the processing negativity of the event-related brain potential response. The processing negativity is an event-related brain potential component that can be recorded in tasks with two types of stimulus inputs presented concurrently, i.e., to be attended stimuli and distracting stimuli. The processing negativity reflects the enhanced processing of the to be attended stimuli in comparison with the distracting stimuli, and as such is a useful neurophysiological indicator of the selectivity of attention.

The ACTH/MSH peptides in those foregoing experiments were administered systemically, i.e., intravenously and orally. Given the evidence that the molecule after intranasal administration directly enters the cerebrospinal fluid, findings of a weakened selective attention also after the intranasal administration of ACTH/MSH-(4–10) would add strong support for the view that these change represent a direct influence of the peptide on the brain. On this background, in a recent study we examined effects of ACTH/MSH-(4–10) on event-related brain potential indicators of selective attention after acute, and in addition after subchronic (over 6 weeks) intranasal administration of the substance (Smolnik et al., 1999). As expected, the single intranasal administration of 1 mg of ACTH/MSH-(4–10) distinctly reduced the processing negativity over anterior cortical areas (Fig. 5). Moreover, after acute intranasal ACTH/MSH-(4–10) subjects were more prone to interference on the Stroop test, which is another test of selective attention. However, both of these effects disappeared after prolonged treatment with the ACTH/MSH-fragment. These findings agree with the results of previous

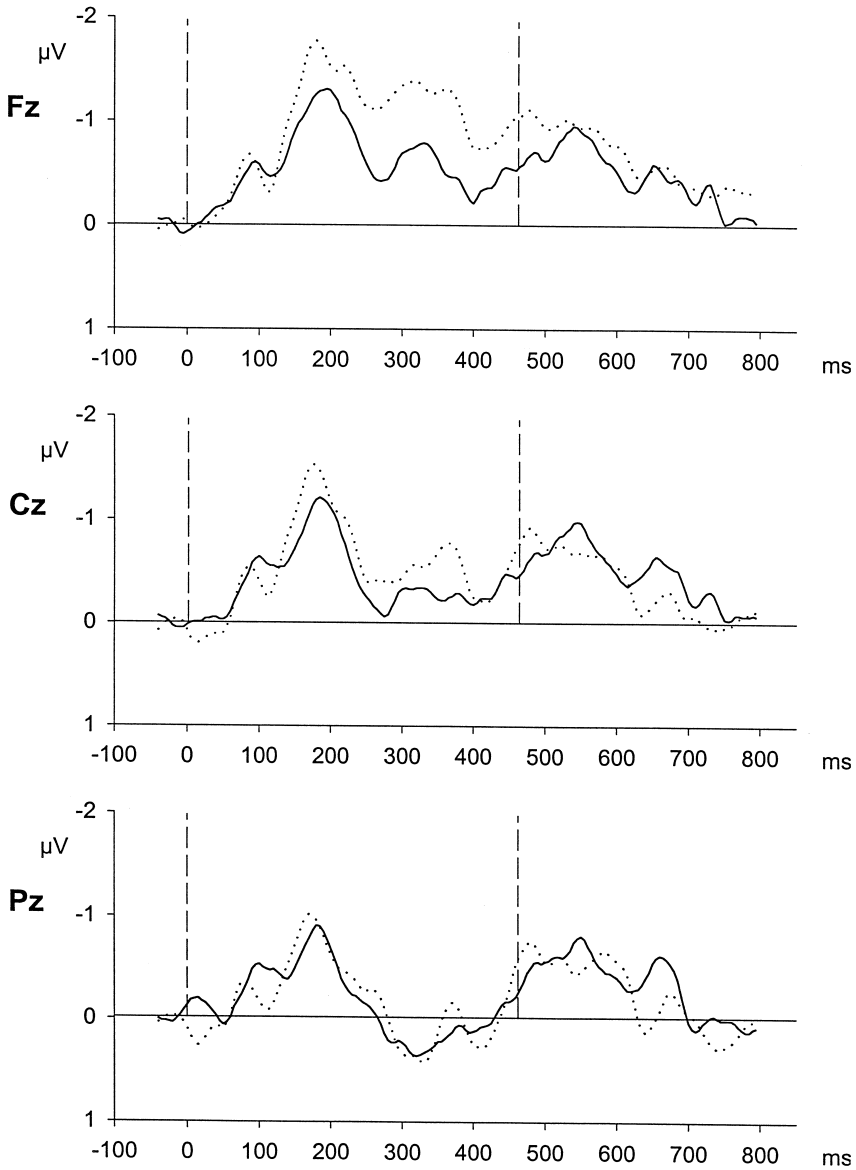

Fig. 5. Processing negativity difference wave recorded from Fz, Cz, and Pz following acute intranasal administration of 1 mg ACTH-(4–10) (—) and following placebo administration session (· · ·). Note reduced processing negativity at anterior recording sites after ACTH-(4–10). Processing negativity amplitude was determined by subtracting the event-related brain potential to standard stimuli when unattended from the event-related brain potential to attended standard pips of the selective attention task 0–460 ms post-stimulus (vertical lines indicate latency bin).

studies in which an intravenous or oral route of peptide administration was employed. Thereby, they provide evidence for a direct action of the substance on attentional functions probably involving frontocortical brain regions. However, effects were revealed after acute administration only. With prolonged treatment with ACTH/MSH-(4–10), additional mechanisms may become activated compensating for the decrease in selective attention.

While the action of ACTH/MSH-molecules on mechanisms of learning and attention has been the matter of research in animals and humans for many years, only recently a key role of melanocortins for the regulation of body weight has been recognised. Body weight and especially body fat stores are known to be rigidly guarded around a set point, whereby long-term homeostasis of fat stores is apparently accomplished by hypothalamic centers that integrate hormonal signals from the periphery such as leptin, insulin and cortisol. These signals interact with a variety of neuropeptides, which belong either to an orexigenic or an anorexigenic network within the hypothalamus. The melanocortin system of the nucleus arcuatus is of major importance within the anorexigenic network. Striking evidence for this has emerged from studies of the agouti protein, which exerts its effects through a competitive antagonism of the natural ligand (probably α-MSH) at the melanocortin receptor. A mutation at the agouti gene locus (A^y) causing ectopic expression of the agouti peptide, leads to a lethal syndrome, which is characterised by

maturity-onset obesity, hyperinsulinemia and hyperglycemia. Among the five subtypes of the melanocortin receptor the melanocortin MC_4 receptor appears to be most closely linked to the regulation of body weight in animals. Thus, genetic deficiency in the melanocortin MC_4 receptor in mice is accompanied by hyperphagia, hyperinsulinemia, hyperglycemia and obesity. Other recent works suggest that defects involving the melanocortin system can lead to obesity also in humans. Krude et al. (1998) identified mutations in the gene for proopiomelanocortin (the precursor of melanocortins) as a cause for a rare human hereditary syndrome featuring severe obesity, red hair, and adrenal insufficiency. It has been supposed that mutations in the gene for melanocortin MC_4 receptor account for 2% to 3% of severe cases of obesity presumably because appetite is not suppressed in these patients by appropriate melanocortin receptor stimulation.

In an animal model of obesity secondary to a genetic deficiency of melanocortin synthesis, treatment with an ACTH/MSH-related agonist of the melanocortin receptor induced dramatic weight loss and normalisation of obesity. This observation led us to suppose that agonists of central nervous melanocortin receptors would reduce body weight also in humans, provided that sufficient amounts of the substance following administration enters the brain. In a first study in normal weight humans, we examined the effects of a 6-week daily treatment with two different melanocortins, ACTH/MSH-(4–10) and desacetyl-α-MSH, on body weight, body fat (assessed by bioelectrical impedance analysis) as well as on plasma concentrations of leptin and insulin. The melanocortins used in the study share all seven amino residues representing the core sequence of melanocortin. Desacetyl-α-MSH may represent one of the natural ligands of the melanocortin MC_4 receptor. In vitro, it was found to exhibit a distinctly greater potency in activating melanocortin MC_4 receptor coupled adenylyl cyclase than ACTH/MSH-(4–10).

Compared with the effects of placebo, the 6-week treatment with ACTH/MSH-(4–10) decreased body fat on average by 1.68 kg ($p < 0.05$) and body weight on average by 0.79 kg ($p < 0.001$, Fig. 6). Decreases in body fat and weight resulted in a diminished body mass index after subchronic ACTH/MSH-(4–10) ($p < 0.001$). Lean body mass and body cell mass both of which index extra-adipose tissue, remained unchanged. Reduction in body fat after administration of ACTH/MSH-(4–10) was associated with a 24% decrease in plasma levels of leptin and with a 20% decrease in plasma insulin concentration.

Although body fat was also slightly reduced after subchronic administration of desacetyl-α-MSH, this effect did not reach significance. There were no changes in any of the other parameters of body composition after desacetyl-α-MSH. Also, desacetyl-α-MSH did not affect concentrations of leptin and insulin.

This data indicate a reducing effect of the melanocortin sequence ACTH/MSH-(4–10) on human body adiposity

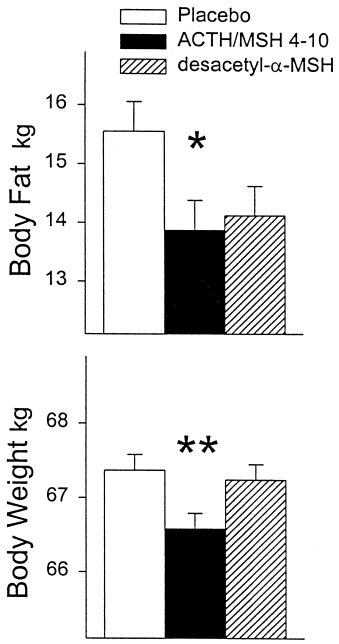

Fig. 6. Body fat and body weight in healthy, normal weight humans after a 6-week period of daily treatment with placebo (empty bars), ACTH-(4–10) (black bars) and desacety-α-MSH (hatched bars). ACTH-(4–10) induced reductions in both measures.

within a rather short treatment period of 6 weeks. Notably, the effect was confirmed by converging results from measures of body weight, bioelectrical impedance analysis of body fat and hormonal measures of leptin and insulin. No side effects occurred and the weekly measurements of body weight indicated a gradual increase in the effect size so that additional weight loss might be expected from longer treatment periods.

Surprisingly, the overall effect of desacetyl-α-MSH on body composition was less distinct than that of ACTH/MSH-(4–10), and failed to reach significance in comparison with the placebo control condition. Desacetyl-α-MSH is the major melanocortin of the rat and human hypothalamus and, in vitro, its potency in activating the melanocortin MC_4 receptor subtype, which is considered the key receptor mediating weight loss, was found to be 300-fold higher than for ACTH/MSH-(4–10). An accelerated in vivo degradation probably cannot account for the failure of desactetyl-α-MSH to reduce body weight in humans, although the kinetics and conversion by brain peptidases so far have not been directly compared between the two melanocortins used here. Rather, the lack of changes in body adiposity after desacetyl-α-MSH could point to an involvement of receptor mechanisms other than melanocortin MC_4 receptor in the effects of ACTH/MSH-(4–10). ACTH/MSH-(4–10) shares many of its neurobehavioral effects (including those on attention in humans) with ORG 2766, a synthetic ACTH/MSH-(4–9) analog with no affinity to the melanocortin MC_4 receptor at all. Accordingly, an as yet unknown receptor type may con-

tribute to the decrease in body fat induced by ACTH/MSH-(4–10). Together, these findings represent a first successful attempt to regulate body weight in humans by means of intranasal peptide administration aiming to directly influence the hypothalamic lipostat.

4. Discussion and conclusions

Results briefly described here, indicate that peptides such as vasopressin, insulin, and ACTH/MSH-(4–10) after intranasal administration are rapidly transported into the cerebrospinal fluid compartment, thereby gaining access to respective central nervous receptors. Presumably, transport from the nasal mucosa to the cerebrospinal fluid is accomplished by passive diffusion across the lamina cribrosa. However, further (active carrier) mechanisms may be also relevant. Also, it is not known whether the peptides once they have reached cerebrospinal fluid become widely distributed to all brain regions or whether certain location (e.g., receptors in close proximity to the olfactory bulb) are preferentially accessed. Despite of these unresolved issues, data provide convergent evidence that the intranasal administration of peptides represents an efficient way around the blood–brain barrier, in order to manipulate directly neuropeptidergic pathways in the human brain. Influences after nasal intake of the peptides pertain to subcortical, hypothalamic functions (sleep processes, regulation of body weight) as well as to cortical functions (memory, selective attention). Moreover, the available evidence suggests a possible exploitation of this approach for clinical purposes, i.e., for the treatment of neuropathological and psychopathological conditions as present, for example, in patients with insomnia, Alzheimer's dementia and obesity. Here, just a few examples have been discussed. The number of peptides, which might exert similarly beneficial effects in the context of brain diseases, is probably much greater. On this background, our observations in humans point to the emergence of a novel approach in neuropharmacology relying on the specific manipulation of peptidergic neurotransmitter systems. In comparison to the classical neurotransmitters, the array of target functions for a neuropeptide is probably smaller and more circumscribed. Also, undesired side effects may be less frequent. In fact, we did not observe any side effect of clinical importance in the studies reported here with vasopressin, insulin and ACTH/MSH-(4–10). Thus, overall data lend themselves to stimulate any attempt to extend the evaluation of intranasal peptidergic treatments to other neuropeptides and other brain diseases.

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