Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
A NOSE–BRAIN PATHWAY FOR PSYCHOTROPIC PEPTIDES: EVIDENCE FROM A BRAIN EVOKED POTENTIAL STUDY WITH CHOLECYSTOKININ

Reinhard Pietrowsky, Andrea Thiemann, Werner Kern, Horst L. Fehm and Jan Born
Department of Clinical Neuroendocrinology, University of Lübeck, Lübeck, Germany

(Received 29 November 1994; in final form 8 August 1995)

SUMMARY

The access of substances to the brain is of particular relevance for the etiology and treatment of psychiatric and neurologic diseases. This study provides functional evidence for a direct access of peptides to the human brain after intranasal administration. Effects were compared of intranasal (IN, 10 μg) and intravenous (IV, 0.25 and 2.5 μg) administered cholecystokinin-8 (CCK) on the auditory event related potential (AERP) in 20 healthy subjects. Also, plasma concentration of cortisol and ACTH were monitored. The study was designed as a placebo-controlled, double-blind within-subject cross-over comparison. AERPs were recorded while the subject performed on an attention task (oddball task). Plasma CCK concentrations after IN administration of CCK were comparable to those after IV administration of 0.25 μg CCK, but were substantially lower than those after 2.5 μg CCK. The P3 complex of the AERP was markedly increased following the IN administration of CCK (p < .01) compared to placebo and to the IV administration of 0.25 μg. This pattern was more obvious in women than men. Increases in plasma ACTH concentrations after CCK reached significance selectively following the IN mode of administration (p < .01). Copyright © 1996 Elsevier Science Ltd

Keywords—Blood–brain-barrier; Nose–brain-barrier; Cholecystokinin; CCK; Auditory evoked potential; P3; Human.

INTRODUCTION

The blood–brain barrier represents an essential obstacle for the pharmacological treatment of human central nervous diseases. Although some evidence exists that substances may enter the brain via the nasal mucosa, little attention has been paid to the nose–brain pathway as a possibility to circumvent the blood–brain barrier. Proteins like horseradish peroxidase and also viruses have been found in substantial amounts in brain areas after their administration to the nasal mucosa (Baker & Spencer, 1986; Balin et al., 1986; Barnett & Perlman, 1993; Eseri & Tomlinson, 1984; Morales et al., 1988). In a previous human study (Pietrowsky et al., 1996) effects of the peptide hormone vasopressin on auditory event-related brain...
potentials (AERPs) were found to be significantly stronger after intranasal (IN) than after intravenous (IV) administration although plasma vasopressin concentrations were comparable for both routes of administration.

Nearly all peptide hormones have been demonstrated to affect aspects of behavior after intracerebroventricular administration in animals indicating a high psychotropic potency for this class of substance. Although changes in central nervous information processing have also been shown in human subjects after IV administration of peptides such as corticotropin (ACTH), vasopressin and cholecystokinin (CCK), the effects in general appeared to be more difficult to demonstrate. This could be ascribed in part to the limited access of blood-borne peptides to the central nervous system. The central nervous effects of peptides after IV administration have been considered to be mediated via stimulation of afferent nerves in the case of CCK (Smith et al., 1981) or via the circumventricular organs, which lack the blood–brain barrier and contain a large number of specific binding sites for peptides such as ACTH or CCK (Weindl & Sofroniew, 1981; Van der Kooy, 1984). Also, for some peptides like vasopressin and insulin, saturable active carrier mechanisms have been detected at the blood–brain barrier (Banks & Kastin, 1987; Pardridge et al., 1985; Zlokovic et al., 1990).

The present experiment aimed to provide further functional evidence for a direct access of peptides to the human brain after IN administration. For this purpose, effects of cholecystokinin on AERPs were compared for the IN and IV routes of administration. CCK is a common peptide hormone in the brain and gut. In mesolimbic–frontocortical neuronal pathways it is co-localized with dopamine (Freeman et al., 1991; Hökfelt et al., 1980; Oeth & Lewis, 1992). Disturbances in these pathways are assumed to account for attentional deficits (Pyccock et al., 1980), which are a crucial symptom of psychosis (Baribeau-Braun et al., 1983). Anti-psychotic effects of CCK and its analogs have been demonstrated (Montgomery & Green, 1988; Nair et al., 1983; Van Ree et al., 1984), however other studies failed (Lotstra et al., 1984; Tamminga et al., 1986). In healthy subjects, CCK-like peptides were found to enhance AERP indicators of attention such as the processing negativity (Pietrowsky et al., 1990, 1993; Schreiber et al., 1995).

Considering the high sensitivity of AERPs to the effects of CCK in those studies, the present experiments focussed on effects of the peptide on the P3 component of the AERP recorded while the subject performed on an attention task. The P3 component is regarded as a complex consisting of the P3 and a subsequent slow wave (Sutton & Ruchkin, 1984). Doses of CCK compared for IN and IV administration were selected based on the results of pilot studies indicating that plasma CCK concentrations were comparable after IV administration of 0.25 μg CCK and after IN administration of 10 μg CCK. To further examine whether changes following CCK were dependent on plasma CCK concentrations, effects of CCK on the AERPs were also tested for a 10-fold higher dose (2.5 μg CCK) administered IV.

METHOD

Subjects

The study was undertaken on 20 students of the medical faculty (10 male, 10 female), aged between 21 and 27 years (mean 23.9 years). Their mean weight was 69.5 kg and their mean height 177.3 cm. Subjects were physically and mentally healthy. In women, the testing occasions took place randomly at the different menstrual cycle phases (luteal, ovulatory, menstrual). Audiological examination excluded any hearing deficiency. Also, rhinitis was
Table 1. Schema of substance administrations

| Treatment          | Placebo | 10 µg CCK intranasally | 0.25 µg CCK intravenously | 2.5 µg CCK intravenously |
|--------------------|---------|------------------------|---------------------------|-------------------------|
| Intranasal:        | Placebo | 10 µg CCK              | Placebo                   | Placebo                 |
| Intravenous:       | Placebo | Placebo                | 0.25 µg CCK               | 2.5 µg CCK              |

excluded. All participants were non-smokers and not under current medication. They had to abstain from caffeinated and alcoholic beverages and food for at least 12 h prior to testing. They had regular sleep-wake rhythms, and it was ascertained that they had slept normally the night before testing. Subjects were informed about the aims of the study and possible side effects of the substance administered. The study was approved by the Committee on Research Involving Human Subjects of the University of Lübeck and each subject gave written consent.

**Procedure and Design**

The study was conducted as a within-subject cross-over comparison, i.e. each subject participated in four test sessions after having received: (1) IN a dose of 10 µg CCK-8; (2) IV a dose of 0.25 µg CCK-8; (3) IV a dose of 2.5 µg CCK-8; and (4) placebo. To blind the subject and the experimenter, the subject received additionally saline solution IV, when CCK was administered IN. When CCK was given IV, saline solution was given, in addition, IN. The placebo treatment consisted of a combined IN and IV administration of saline solution (Table 1).

The order of treatments was balanced according to a Latin square (i.e. five subjects—three males, two females—received 10 µg CCK IN first; five subjects—two males, three females—received 0.25 µg CCK IV first; five subjects—two males, three females—received 2.5 µg CCK IV first; and the remaining five subjects—three males, two females—received placebo at their first testing occasion). CCK-8 was used since, unlike CCK-33, it is commercially available for use in humans. CCK-8 is the active fragment of the molecule with identical biological actions.

For IN administration, CCK-8 (Sigma, Switzerland) was dissolved in sterile water, and a dose of 5 µg (contained in one puff of 100 µl) was sprayed in each nostril. For IV infusion, CCK-8 (Kinevac®, Squibb, USA) was dissolved in 100 ml saline solution and administered as a constant rate infusion within 30 min.

Experimental sessions took place in a sound attenuated and electrically shielded room with the subject sitting in a reclining chair. They were scheduled at 0900h, 1000h, and 1100h. In the beginning of the experimental sessions the IN treatment was administered and simultaneously IV infusions began. Recordings of AERPs started 30 min later and lasted for about 15 min. AERPs were obtained while the subject performed an auditory oddball task. On this task, subjects attended to a sequence of 400 tone pips (duration: 60 ms, intensity: 64 dB SPL) presented binaurally via headphones. These pips were either standard pips (80% probable, pitch: 1000 Hz) or rare target pips (20% probable, pitch: 1064 Hz).
randomly interspersed among the frequent standard pips. Interstimulus intervals varied randomly between 1 and 3 s (average 2 s). Subjects were instructed to press as fast and as accurately as possible a button (with the thumb of the dominant hand), whenever a target pip occurred. They were also instructed to fixate their gaze on a dot located centrally in front of them, and to avoid eye blinks and body movements during task performance. Prior to their first experimental session, subjects practiced the task with a shortened series of 40 tone pips.

For blood sampling a catheter was placed in the vena cephalica contralateral to that used for IV administration of treatments. To avoid clotting, 100 ml saline solution was slowly infused through this catheter within 60 min. To determine plasma hormone concentrations, blood samples were collected immediately prior to administration of treatments, and 10, 20, 30, and 45 min later. The sample collected 30 min following administration of the IN treatment (and following the start of the IV infusion), was immediately before presentation of the oddball task.

Recordings of AERPs and Hormone Assays

During the subject’s performance on the oddball task, EEG-recordings (5 s time constant, low pass filter: 70 Hz, -12 dB/octave) were obtained from non-polarizable electrodes (Ag/AgCl, 16 mm diameter, Beckman Instruments, USA) attached along the midline at Fz, Cz, and Pz. Linked electrodes at the mastoids of the right and left ear served as reference. The ground electrode was attached to the forehead. For detection of eye movement artifacts, the vertical electro-oculogram (EOG) was recorded from electrodes above and below the left eye. EEG and EOG signals were amplified by a Nihon Kohden Neurofax amplifier and digitized (CED 1401, Cambridge Electronic Design, UK) at a rate of 200 Hz and stored on a computer disk for off-line averaging of AERPs.

Blood samples were centrifuged immediately after collection and frozen at -20°C for later determination of plasma levels of CCK-8 using the G-160 antibody (sensitivity: 0.75 pmol/l; intra- and interassay coefficients of variation: 10–15%; Höcker et al. (1992)). Also, ACTH and cortisol were determined to evaluate a possible potency of CCK to stimulate secretory activity of the pituitary–adrenal-system (Späth-Schwalbe et al., 1988). Both hormones were also measured by radioimmunoassay: ACTH (Euro-Diagnostics BV, Apeldoorn, The Netherlands; sensitivity: 0.88 pmol/l, the intra-assay coefficient of variation ranged from 10% at 3.3 pmol/l to 2% at 44 pmol/l), and Cortisol (Biermann GmbH, Bad Nauheim, Germany; sensitivity: 4.7 nmol/l, intra-assay coefficient of variation < 5% between 27.6 nmol/l and 414 nmol/l). Samples from an individual subject were analyzed in duplicate in the same assay.

Data Reduction and Analysis

Individual AERPs were averaged separately for each subject and separately for experimental condition, which were: treatment (10 µg CCK-8 IN, 0.25 µg CCK-8 IV, 2.5 µg CCK-8 IV, placebo); type of tone pip (standard, target); and electrode site (Fz, Cz, Pz). The averaging period covered a 200 ms pre-stimulus baseline and an 800 ms post-stimulus interval. Periods were excluded from analysis if they contained blinks, gross eye movements, or other potentials exceeding 150 µV.

Determination of the P3 complex and its subcomponents (P3, slow wave) relied on areas under the curve (AUC), which were calculated (with reference to the average potential during the pre-stimulus baseline) for the 280–700 ms post-stimulus latency interval (P3 complex) and separately for the 280–500 ms (P3) and 500–700 ms (slow wave) latency.
Intranasal Versus Intravenous CCK

Intranasal Versus Intravenous CCK intervals. For an additional evaluation of the N1 and P2 components of the AERP, of the peak amplitude of P3 and for determination of latencies (with reference to stimulus onset) of these components, peak-to-baseline amplitudes were determined within latency bins accounting for the N1 (70–140 ms post-stimulus), P2 (130–230 ms) and P3 (280–700 ms).

Statistical evaluation of the AERP measures was based on analyses of variance (ANOVA). ANOVA included repeated measures factors for the treatment conditions and electrode sites. Effects of the treatments were specified by post-hoc pairwise comparisons between any two of the treatment conditions, and separately for each electrode location. Presentation of the results was concentrated on the electrode site where the respective component displayed its maximum since at other electrode sites it could be contaminated by other components. Treatment effects on plasma hormone concentrations were evaluated by analyses of covariance (ANCOVA) with the concentration prior to administration of treatments as a covariate. Additionally, all analyses were run with a grouping factor for sex to test for differences between the gender of the subjects. A Greenhouse–Geisser corrected p-value < .05 was considered significant. The level of significance for post-hoc pairwise comparisons was Bonferroni-adjusted to the number of comparisons performed. Behavioral measures (reaction time to targets and false reactions) were evaluated by non-parametric statistics (Wilcoxon t-test) for differences between each two treatments.

RESULTS

Plasma CCK-8 Levels

Both, the IN administration of 10 μg CCK, as well as the IV infusion of 0.25 μg CCK increased plasma CCK levels slightly but significantly (Fig. 1). The increase for these two treatments was very similar, and no significant differences in plasma CCK concentrations were observed at any time of measurement. By contrast, the IV infusion of 2.5 μg CCK induced a pronounced rise in plasma CCK concentrations, significantly exceeding those after IN CCK and 0.25 μg IV CCK at 10 and 20 min after starting the infusion. In all treatment conditions, no significant differences in plasma CCK between the sexes were observed.

Plasma ACTH and Cortisol Levels

Plasma ACTH levels in general were significantly higher in males than in females (F(1,17) = 26.3, p < .001). Following the IN CCK treatment, plasma ACTH levels were enhanced compared to the placebo condition (mean ± SEM: 6.6 ± 0.8 pmol/l; 5.2 ± .6 pmol/l; F(1,17) = 9.9, p < .01; Table II). Effects after IV infusion of 0.25 μg (6.0 ± 0.8 pmol/l) and of 2.5 μg CCK (5.3 ± 0.7 pmol/l) did not reach significance. Plasma cortisol levels did not differ significantly between the experimental conditions.

AERP-measures

Since effects on the P3 complex were more pronounced for AUC than for amplitude measures, the report of P3 related results is restricted to the AUC measures. The P3 complex as measured by the total area under the curve between 280–700 ms displayed its maximum at Pz. P3 complex at Pz was generally increased following IN and IV administration of CCK (F(3,54) = 5.6, p < .05, for main effect of treatment; Fig. 2). However, pairwise statistical comparisons with the effects of placebo confirmed a significant enhancement of the P3 complex only for the IN route of administration (F(1,18) = 16.5, p < .01; Fig. 3, Table III). In addition, P3 was generally larger for women than for men (F(1,18) = 21.5, p < .001).
Fig. 1. Plasma CCK-levels (pmol/l) prior to and at 10, 20, and 30 min following the administration of 10 µg CCK intranasally (thin solid line), 2.5 µg CCK intravenously (dashed line) and 0.25 µg CCK intravenously (thick solid line).

Table II. Plasma ACTH and cortisol measures

|               | Placebo | 10 µg CCK IN | 0.25 µg CCK IV | 2.5 µg CCK IV |
|---------------|---------|--------------|---------------|--------------|
| **ACTH (pmol/l)** |         |              |               |              |
| Baseline      | 8.07 (1.1)| 7.44 (0.9)  | 7.66 (1.1)    | 6.56 (0.8)   |
| 10 min        | 6.18 (0.7)| 7.56 (1.0)  | 6.95 (1.0)    | 5.54 (0.7)   |
| 20 min        | 5.37 (0.6)| 6.89 (0.9)  | 6.12 (0.8)    | 5.21 (0.6)   |
| 30 min        | 4.80 (0.5)| 6.25 (0.7)  | 5.70 (0.7)    | 5.10 (0.6)   |
| 45 min        | 4.60 (0.6)| 5.59 (0.7)  | 5.08 (0.6)    | 5.50 (0.9)   |
| **Cortisol (nmol/l)** |       |              |               |              |
| Baseline      | 480 (33.1)| 477 (30.4)  | 475 (33.1)    | 444 (30.4)   |
| 10 min        | 431 (30.4)| 431 (30.4)  | 420 (33.1)    | 397 (27.6)   |
| 20 min        | 395 (30.4)| 420 (30.4)  | 389 (30.4)    | 373 (27.6)   |
| 30 min        | 373 (30.4)| 406 (30.4)  | 381 (30.4)    | 362 (27.6)   |
| 45 min        | 348 (27.6)| 370 (24.8)  | 348 (24.8)    | 315 (24.8)   |

Mean (± SEM) plasma ACTH and cortisol levels following the administration of placebo, 10 µg CCK IN, 0.25 µg CCK IV and 2.5 µg CCK IV measured prior to the administration of the substances (baseline) and at 10, 20, 30, and 45 min after the administration of the substances.

Separate evaluation of the P3 (280–500 ms post-stimulus) and slow wave (500–700 ms post-stimulus) subcomponents of the P3 complex indicated that the effects of CCK concentrated on the slow wave. Concerning the P3 subcomponent, the main effect for treatment reached significance across all four treatment conditions ($F(3,54) = 3.2, p < 0.05$), possibly due to the fact that P3 was generally higher during the three CCK conditions than during placebo. However, pairwise comparisons between the effects of placebo and any of the three CCK conditions failed to reach the 5% level of significance.
Intranasal Versus Intravenous CCK

Fig. 2. Difference waveform of the mean AERP (µV) for the standard stimuli and the target stimuli at recordings from Pz (between 200 ms pre-stimulus and 800 ms post-stimulus). This difference waveform displays the P3 complex. Vertical lines indicate the interval in which the P3 complex and its subcomponents were determined. Negativity is upward.

Fig. 3. Area under the curve (AUC, means ± SEM) for the P3 complex between 280–700 post-stimulus. Intranasal administration of 10 µg CCK significantly enhanced P3 complex AUC compared to placebo and compared to intravenous administration of 0.25 µg CCK. *p < .05, **p < .01.

The slow wave was also generally larger following CCK treatments than following placebo ($F(3,54) = 6.0$, $p < .01$, for the main effect of treatment). Subsequent pairwise comparisons with placebo confirmed significantly enhancing effects of IV administration of 2.5 µg CCK ($F(1,18) = 6.7$, $p < .05$) and of the IN administration of CCK ($F(1,18) = 18.7$, $p < .001$). The increase in slow wave after IN administration of CCK was also significantly
Table III. AERP measures

|                      | Placebo | 10 µg CCK IN | 0.25 µg CCK IV | 2.5 µg CCK IV |
|----------------------|---------|--------------|----------------|--------------|
| N1 amplitude (µV)    |         |              |                |              |
| men                  | -7.17 (0.7) | -7.12 (0.6) | -7.62 (0.8) | -6.98 (0.8) |
| women                | -8.92 (1.3) | -9.56 (1.5) | -8.92 (1.4) | -9.94 (1.5) |
| P2 amplitude (µV)    |         |              |                |              |
| men                  | 6.08 (0.6) | 7.26 (0.7)  | 7.25 (0.9)    | 7.36 (0.9)  |
| women                | 8.47 (1.0) | 6.68 (1.2)  | 8.77 (1.0)    | 7.84 (1.0)  |
| P3 amplitude (µV)    |         |              |                |              |
| men                  | 6.97 (0.9) | 8.27 (0.8)  | 7.78 (1.0)    | 8.58 (1.3)  |
| women                | 15.33 (1.9) | 17.23 (1.7) | 16.60 (2.0) | 16.11 (1.8) |
| P3-complex AUC (mV*ms) |         |              |                |              |
| men                  | 1.49 (0.2) | 1.87 (0.2)  | 1.66 (0.2)    | 1.93 (0.3)  |
| women                | 3.20 (0.5) | 4.46 (0.5)  | 3.96 (0.5)    | 3.78 (0.4)  |
| P3 AUC (mV*ms)       |         |              |                |              |
| men                  | 0.84 (0.2) | 1.10 (0.1)  | 1.05 (0.1)    | 1.14 (0.2)  |
| women                | 2.31 (0.4) | 2.81 (0.3)  | 2.65 (0.3)    | 2.53 (0.3)  |
| Slow wave AUC (mV*ms) |         |              |                |              |
| men                  | 0.65 (0.2) | 0.77 (0.1)  | 0.61 (0.2)    | 0.79 (0.2)  |
| women                | 0.89 (0.2) | 1.65 (0.2)  | 1.31 (0.2)    | 1.25 (0.1)  |

Mean (± SEM) amplitudes (µV) of the N1 and P2 (at Cz), P3, and the area under the curve (AUC, mV*ms) of the P3-complex and P3 and slow wave subcomponents (at Pz), following the administration of placebo, 10 µg CCK IN, 0.25 µg CCK IV and 2.5 µg CCK IV in healthy men and women.

higher when compared to the effect of IV infusion of 0.25 µg CCK (F(1,18) = 8.7, p < .05; Fig. 4, top panel).

Apart from the significance of the main effect of treatment (across the subjects of both sexes) the increase of the slow wave was substantially more pronounced in women than in men, as confirmed by a significance for the treatment × sex interaction term (F(3,54) = 3.6, p < .05). In subsequent pairwise comparisons with placebo the treatment × sex interaction reached significance also for the effects of IN administration of CCK (F(1,18) = 10.0, p < .05) but not for the effects of CCK after IV infusion. In subsequent comparisons, performed separately for the women and for the group of men, effects of IN CCK (versus placebo) reached statistical significance in the women but not the men (F(1,9) = 20.4, p < .01; Fig. 4, bottom).

Amplitudes of N1 and P2 as well as latencies of N1, P2 and P3 were not affected by any of the CCK treatments (Table III).

Behavioral Measures

Reaction time to the detection of targets and the number of false reactions in the target detection did not differ significantly between the treatments. Also, the treatments did not differ in self-perceived activation or mood, as assessed by the EWL, a standardized German adjective list. Subjects could also not consistently identify whether they had received placebo, IN CCK or IV CCK.
Intranasal Versus Intravenous CCK

Slow wave

Fig. 4. Top: Area under the curve (AUC, means ± SEM) for the slow wave subcomponent (500–700 ms post-stimulus) of the P3 complex. Compared to placebo, the slow wave AUC was significantly enhanced following intravenous administration of 2.5 µg CCK and the intranasal administration of 10 µg CCK. The increase in slow wave AUC following intranasal administration was also greater than following intravenous administration of 0.25 µg CCK. Bottom: The differences in slow wave AUC with reference to the effects of placebo are indicated for the conditions of intranasal administration of 10 µg CCK, of intravenous administration of 0.25 µg CCK and of intravenous administration of 2.5 µg CCK, separately for the group of men (white bars) and women (hatched bars). In women, but not in men, the slow wave AUC was significantly increased following intranasal administration of CCK. *p < .05, **p < .01.
DISCUSSION

The present study compared effects of CCK following an IN and IV route of administration on brain evoked potentials. Plasma concentrations of CCK after IN administration of 10 μg were comparable with those induced by an IV administration of 0.25 μg CCK. Yet, P3 complex was significantly increased after IN administration of CCK, whereas effects for the IV route were weaker and failed to reach significance. Moreover, the effect of CCK concentrating on the late slow wave part of the P3 complex following the IN administration was even significantly larger than that after 0.25 μg CCK IV. Increasing the dose of IV administered CCK to 2.5 μg did strengthen the central nervous effects of CCK restricted on the slow wave, although plasma CCK concentrations were markedly enhanced. Also, only after IN but not after IV administration of CCK, a slight but significant increase in ACTH concentration was observed. The effects of CCK after IN administration, exceeding those after IV administration, suggest that the peptide has a direct access to the brain after IN administration, without entering the blood stream.

Since N1 and P2 were not affected by the IN CCK treatment, it is suggested that this treatment affects only controlled stimulus processing, in contrast to automatic processing as reflected by the N1 and P2. Likewise, the fronto–centrally distributed P3a, indicating orienting, was not affected by CCK. It should be noted, that the P3 component described in the present paper refers to the P3b which has a centro–parietal distribution, in contrast to the P3a, which has a fronto–central distribution and habituates rapidly with stimulus repetition.

The finding of an enhanced P3 complex after CCK confirms results from a foregoing study (Dodt et al., personal communication) indicating a similar enhancement after IV administration of the CCK-analog ceruletide, which is assumed to possess a higher potency than CCK (Jurna & Zetler, 1981). Like in the present study, effects of ceruletide concentrated on the slow wave component of the P3 complex, which has been considered a reflection of further cortical processing of the task-relevant target stimuli (Johnson & Donchin, 1985). Effects in this study were also more pronounced in women than men. Although this effect may be attributed to the smaller body mass of the women, the failure to demonstrate differences in plasma CCK-levels between the sexes and the independence of the P3 increase from circulating CCK argue against this interpretation. Rather, the women’s brain seems to be more sensitive to effects of CCK. Accordingly, central nervous effects of CCK are modulated by estrogens (Karlsson et al., 1992; Oro et al., 1988).

Most important, the increase in P3 following the IN administration of CCK, was significantly more pronounced than that following the IV administration of CCK, when analyzed for subjects of both sexes and for women only. This further supports the notion, that the enhancing effect of IN CCK on P3, which is much more pronounced in women, is independent from circulating CCK. Since the P3 complex was generally larger in women than in men, the results rather suggest that the enhancing effect of IN CCK may depend on the existence of a large P3 prior to treatment, i.e. an increased activity of the neuronal systems generating the P3 complex.

The enhancement of ACTH following the IN treatment, although significant, appeared to be too small to manifest itself in a concomitant increase in cortisol secretion. Despite its small rise, this effect is in accordance with results from animal and human studies indicating a stimulating effect of CCK on secretory activity of the pituitary–adrenal system (Späth-Schwalbe et al., 1988). Again, it further hints to a direct central nervous effect of IN administered CCK, since the stimulating effect of CCK on ACTH did not occur following the IV administrations of CCK.
Demonstrating a facilitated access of CCK for an influence on brain functions after IN administration of the peptide, the present data provide functional evidence for the notion of a nose–brain-pathway, which has been derived from animal experiments. Several studies in rats have conclusively demonstrated a substantial brain uptake of radioactive labeled proteins, like horseradish peroxidase after application to the nasal or olfactory mucosa (Balín et al., 1986). Moreover, infections of brain tissue, like Borna disease (Shankar et al., 1992) and herpes simplex encephalitis (Eseri & Tomlinson, 1984; Stroop et al., 1984), may result from viruses entering the brain via the olfactory mucosa.

Several mechanisms have been considered that transport molecules from the nose to the brain. Proteins and viruses were found to be taken up at the axons of the olfactory system and moved towards the brain via anterograde spread (Barthold, 1988; Morales et al., 1988; Perlman et al., 1990). However, passage via this route is time consuming and takes up to several days, thus excluding that this mechanism may account for the present effects after IN CCK. Alternatively, proteins may pass through intercellular clefts in the olfactory epithelium to reach the olfactory axons, and to diffuse along these axons into the central nervous system, as has been demonstrated for horseradish peroxidase (Balín et al., 1986). Substances entering the brain via this route may pass within 45 min from the nose to the brain (Balín et al., 1986). Thus, extracellular diffusion could also be the basis of the present effects of IN CCK on AERPs which occurred within 45 min after IN administration of the peptide.

Alternatively to the main olfactory system, substances may influence central nervous processes via the accessory olfactory system, i.e. the vomeronasal organ (VNO). The VNO, which is present in most adult people (García-Velasco & Mondragon, 1991), is a chemosensory organ differing in morphology and neuroanatomical connections from the olfactory system. It is particularly involved in the transduction of pheromones (Monti-Bloch et al., 1994) to the CNS and in the regulation of sexual behavior, mainly via LHRH, which is contained in its afferent nerves (Meredith & Fernandez-Fewell, 1994). While peptidergic information may be transduced via specific receptors in the VNO, an axonal transport of peptides along the nerves of the VNO, as described for the olfactory system, may also occur and is already shown for horseradish peroxidase (Itaya, 1987). Until now, no specific receptors for CCK-like substances have been demonstrated in the VNO. However, since the CCK-like substance ceruletide is present in amphibian skin, a phylogenetic role of ceruletide as a pheromone can not be excluded. Thus, as a phylogenetic relict, receptors for ceruletide may be present in the human VNO, which also bind to CCK-8.

The present data do not exclude that the increase in P3 after IN administration is partly due to a brain uptake of blood-borne CCK. Evidence exists that systemic CCK may act across the blood–brain barrier via the circumventricular organs, like the area postrema, containing a large number of specific binding sites for CCK (Carter & Lightman, 1987; Van der Kooy, 1984) and via vagal afferents (Mercer & Lawrence, 1992; Smith et al., 1981). Nevertheless, the effect of CCK after IV administration being significantly smaller than after IN administration suggests a limited uptake of the peptide from systemic blood. However, a saturable transduction of information across the blood–brain barrier about peripheral CCK-levels can also occur by a limited receptor mediated carrier mechanism involving second messengers at the circumventricular organs or at vagal afferents (Carter & Lightman, 1987; Smith & Gibbs, 1984).

It cannot be more than speculated, on how CCK affects brain functioning after having entered the brain. It is conceivable that CCK directly diffuses to receptor sites of the basal
forebrain or may act on receptors in the olfactory bulb from which efferent nervous transmission leads to limbic and forebrain structures. These areas have been found to be rich in high affinity binding sites for CCK-like peptides (Bouras et al., 1986; Dietl et al., 1987) and also play an important role in the regulation of attentional processes (Pribram & Luria, 1973; Stuss & Benson, 1986; Woods & Knight, 1986). The present increase of the P3 amplitude, together with previously reported AERP changes after CCK (Pietrowsky et al., 1993; Schreiber et al., 1995), indicate an improving effect of CCK on these attentional processes.

It is noteworthy in this context that endogenous CCK is co-localized with dopamine in neurons of the mesolimbic–frontocortical dopaminergic system, which has been considered to be involved in the pathogenesis of attentional deficits in schizophrenic patients (Baribeau-Braun et al., 1983). Trials to improve schizophrenic symptoms by treatment with CCK have yielded inconclusive results (for a review: Montgomery & Green, 1988). Yet all of these studies used the IV route of administration. Thus, in the light of the present findings of a facilitated access of IN CCK to the brain, an attempt may be undertaken to improve attentional functions in schizophrenic patients via IN administration of the peptide.

Acknowledgements: We are grateful to S. Baxmann, A. Otterbein, and B. Fink for technical assistance. The study was supported by a grant from the DFG to H.L.F. and J.B.

REFERENCES

Baker H, Spencer RF (1986) Transneuronal transport of peroxidase-conjugated wheat germ agglutinin (WGA-HRP) from the olfactory epithelium to the brain of the adult rat. Exp Brain Res 63:461–473.
Balin BJ, Broadwell RD, Salcman M, El-Kalliny M (1986) Avenues for entry of peripherally administered protein to the central nervous system in mouse, rat, and squirrel monkey. J Comp Neurol 251:260–280.
Banks WA, Kastin AJ (1987) Saturable transport of peptides across the blood–brain barrier. Life Sci 41:1319–1338.
Baribeau-Braun J, Picton TW, Gosselin J (1983) Schizophrenia: A neurophysiological evaluation of abnormal information processing. Science 219:874–876.
Barnett EM, Perlman S (1993) The olfactory nerve and not the trigeminal nerve is the major site of CNS entry for mouse hepatitis virus, strain JHM. Virology 194:185–191.
Barthold SW (1988) Olfactory neural pathway in mouse hepatitis virus nasoencephalitis. Acta Neuropath (Berlin) 76:502–506.
Bouras C, Magistretti PJ, Morrison JH (1986) An immunohistochemical study of six biologically active peptides in the human brain. Hum Neurobiol 5:213–226.
Carter DA, Lightman SL (1987) A role for the area postrema in mediating cholecystokinin-stimulated oxytocin secretion. Brain Res 435:327–330.
Dietl MM, Probst A, Palacios JM (1987) On the distribution of cholecystokinin receptor binding sites in the human brain: an autoradiographic study. Synapse 1:169–183.
Eseri MM, Tomlinson AH (1984) Herpes simplex encephalitis. J Neurol Sci 64:213–217.
Freeman AS, Chiodo LA, Lentz SI, Wade K, Bannon MJ (1991) Release of cholecystokinin from rat midbrain slices and modulatory effect of D2 DA-receptor stimulation. Brain Res 555:281–287.
García-Velasco J, Mondragon M (1991) The incidence of the vomeronasal organ in 1000 human subjects and its possible clinical significance. J Steroid Biochem Mol Biol 39:561–563.
Höcker M, Schmidt WE, Creuzfeldt W, Choudhury AR, Nustede R, Schafmayer A, Fölsch UR (1992) Determination of plasma cholecystokinin (CCK) concentrations by bio-assay and radioimmunoassay in man. A critical evaluation. Regul Pept 37:255–269.
Höpfelt T, Rehfell JD, Skirboll L, Ivermark B, Goldstein M, Markey K (1980) Evidence for coexistence of dopamine and CCK in mesolimbic neurons. Nature 285:476–478.
Itaya SK (1987) Anterograde transynaptic transport of WGA-HRP in rat olfactory pathways. Brain Res 409:205–214.
Johnson R Jr, Donchin E (1985) Second thoughts: Multiple P3s to emotional stimuli and their theoretical significance. Psychophysiology 23:684–694.

Jurna I, Zetler G (1981) Antinociceptive effect of centrally administered caerulein and cholecystokinin octapeptide (CCK-8). Europ J Pharmacol 73:323–331.

Karlsson R, Lindén A, von Schoultz B (1992) Suppression of 24-hour cholecystokinin secretion by oral contraceptives. Am J Obstetrics Gynecol 167:58–59.

Lotstra F, Verbanck P, Mendlewicz J, Vanderhaeghen JJ (1984) No evidence of antipsychotic effect of caerulein in schizophrenic patients free of neuroleptics: a double-blind cross-over study. Biol Psychiatry 19:877–882.

Mercer JG, Lawrence CB (1992) Selectivity of cholecystokinin (CCK) receptor antagonists, MK-329 and L-365,260, for axonally-transported CCK binding sites on the rat vagus nerve. Neurosci Lett 137:229–231.

Meredith M, Fernandez-Fewell G (1994) Vomeronasal system, LHRH, and sex behavior. Psychoneuroendocrinology 19:657–672.

Montgomery SA, Green MCD (1988) The use of cholecystokinin in schizophrenia: a review. Psychol Med 18:593–603.

Monti-Bloch L, Jennings-White C, Dolberg DS, Berliner DL (1994) The human vomeronasal system. Psychoneuroendocrinology 19:673–686.

Morales JA, Herzog S, Kompter C, Frese K, Rott R (1988) Axonal transport of Borna disease virus along olfactory pathways in spontaneously and experimentally infected rats. Med Microbiol Immunol 177:51–68.

Nair NPV, Bloom DM, Nestoros JN, Schwartz G (1983) Therapeutic efficacy of cholecystokinin in neuroleptic-resistant schizophrenic subjects. Psychopharmacol Bull 19:134–136.

Ooth KM, Lewis DA (1992) Cholecystokinin- and dopamine-containing mesencephalic neurons provide distinct projections to monkey prefrontal cortex. Neurosci Lett 145:87–92.

Oro AE, Simerly RB, Swanson LW (1988) Estrous cycle variations in levels of cholecystokinin immunoreactivity within cells in three interconnected sexually dimorphic forebrain nuclei. Neuroendocrinology 47:225–235.

Partridge WM, Eisenberg J, Yang J (1985) Human blood–brain barrier insulin receptor. J Neurochem 44:1771–1778.

Perlman S, Evans G, Afifi A (1990) Effects of olfactory bulb ablation on spread of a neurotropic coronavirus into the mouse brain. J Exp Med 172:1127–1132.

Pietrowsky R, Fehm HL, Er A, Bathelt B, Born J (1990) Influences of the cholecystokinin analog ceruletide on human sleep and evoked potentials. Neuropsychobiology 23:41–47.

Pietrowsky R, Schiemann C, Fehm HL, Born J (1993) The P2 amplitude of the auditory evoked potential is diminished by the cholecystokinin-analog ceruletide. J Psychophysiol 7:3–10.

Pietrowsky R, Strüben C, Mölle M, Fehm HL, Born J (1996) Brain potential changes after intranasal versus intravenous administration of vasopressin: Evidence for a direct nose–brain pathway for peptide effects in man. Biol Psychiatri 39:241–250.

Pribram KH, Luria AR (1973) Psychophysiology of the Frontal Lobes. Academic Press, New York.

Pycock CH, Carter CJ, Kerwin RW (1980) Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on neurotransmitter systems in subcortical sites in the rat. J Neurochem 34:91–99.

Schreiber H, Stolz-Born G, Pietrowsky R, Kornhuber HH, Fehm HL, Born J (1995) Improved event-related potential signs of selective attention after the cholecystokinin-analog ceruletide in healthy controls. Biol Psychiatri 37:702–712.

Shankar V, Kao M, Hamir AN, Sheng H, Koprowsi H, Ditzen-schold B (1992) Kinetics of virus spread and changes in levels of several cytokine mRNAs in the brain after intranasal infection of rats with Borna disease virus. J Virology 66:992–998.

Smith GP, Gibbs J (1984) Gut peptides and postprandial satiety. Fed Procceed 43:2889–2892.

Smith GP, Jerome C, Cushin BJ, Eterno R, Simansky KJ (1981) Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science 213:1036–1037.

Späth-Schwabale E, Pieroth L, Pietrowsky R, Born J, Fehm HL (1988) Stimulation of the pituitary adrenocortical system in man by cerulein, a cholecystokinin-8-like peptide. Clin Physiol Biochem 6:316–320.

Stroop WG, Rock DL, Fraser NW (1984) Localization of herpes simplex virus in the trigeminal and olfactory systems of the mouse central nervous system during acute and latent infection. Lab Invest 51:27–38.
Stuss DT, Benson DF (1986) The Frontal Lobes. Raven Press, New York.
Sutton S, Ruchkin DS (1984) The late positive complex. In: Karrer R, Cohen J, Tueting P (Eds) Brain
and Information: Event-related Potentials, New York Academy of Science, New York, pp 1–23.
Tamminga CA, Littman RL, Alphs LD, Chase TN, Taker GK, Wagman AM (1986) Neuronal
cholecystokinin and schizophrenia: pathogenic and therapeutic studies. Psychopharmacology
88:387–391.
Van der Kooy D (1984) Area postrema. Site where cholecystokinin acts to decrease food intake. Brain
Res 295:345–347.
Van Ree JM, Verhoeven WMA, Brouwer GH, De Wied D (1984) Ceruletide resembles antipsychotics
in rats and schizophrenic patients. Neuropsychobiology 12:4–8.
Weindl A, Sofroniew MV (1981) Relation of neuropeptides to mammalian circumventricular organs.
In: Martin JB, Reichlin S, Bick KL (Eds) Neurosecretion and Brain Function. Raven Press, New
York, pp 303–320.
Woods DL, Knight RT (1986) Electrophysiologic evidence of increased distractability after
dorsolateral pre-frontal lesions. Neurology 36:212–216.
Zlokovic BV, Segal MB, Davson H, Lipovac MN, Hyman S, McComb JG (1990) Circulating
neuroactive peptides and the blood brain and blood cerebrospinal fluid barrier. Endocrinol Exp
24:9–17.