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Dose–response relationships of intranasal cholecystokinin and the P300 event-related brain potential

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Abstract

The gut and brain peptide cholecystokinin (CCK) has been found to improve controlled stimulus processing and attention as indicated by the late positive complex (LPC) of the event-related brain potentials (ERPs). A direct nose–brain pathway for cortical effects of intranasally administered CCK-8S has been described, although the precise transmission within this pathway is still unknown. The present study compared the effects of two doses of CCK-8S (10 and 20 μg) and placebo after intranasal administration on the LPC of the ERP in healthy male and female subjects to further elucidate mechanisms of this nose–brain pathway. ERPs were recorded in an oddball-paradigm. Results showed that both doses of CCK-8S induced a positive shift of the ERP. This effect did not differ between the 10 and 20 μg dosage of CCK-8S. The results indicate that a saturable mechanism may be responsible for the transmission of CCK-8S from the nose into the brain, since both doses of CCK-8S induced comparable increases of the ERP subcomponents. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Cholecystokinin; Intranasal administration; Event-related potentials; LPC; P300; Nose–brain pathway

1. Introduction

Cholecystokinin (CCK) is a well-characterized peptide hormone initially discovered in the gut and subsequently localized in the central nervous system. In mesolimbic–frontocortical neuronal pathways, it is colocalized with dopamine (DA; Freeman et al., 1991; Hökfelt et al., 1980; Oeth and Lewis, 1992). Dysfunctions in these pathways are considered essential for the pathology of psychotic behavior, especially accounting for controlled processing and attentional deficits (Abelson, 1995; Beinfeld and Garver, 1991; Baribeau-Braun et al., 1983; Pycock et al., 1980). Several studies demonstrated the antipsychotic effects of CCK and its analogs (Fink et al., 1998; Montgomery and Green, 1988; Van Ree et al., 1984; Nair et al., 1983). Also, CCK-like peptides (i.e., CCK-8S, a sulphated octapeptide fragment of CCK) enhanced brain indicators of selective attention such as processing negativity in healthy subjects (Pietrowsky et al., 1997; Schreiber et al., 1995).

Most of the described central nervous effects of peripherally administered CCK-8S are considered to be mediated via peripheral receptors located at vagal afferences or at the area postrema (Hyde and Peroutka, 1989), although centrally located CCK receptors may also contribute to these effects (Hill et al., 1987). Recent studies in humans indicate a direct nose–brain pathway showing that the intranasal administration of CCK and other peptides such as insulin and corticotropin-releasing hormone (CRH) have stronger central nervous effects than a comparable intravenous administration (Kern et al., 1997, 1999; Pietrowsky et al., 1996, 2001). At least two general mechanisms for a nose–brain transmission can be differentiated: receptor-independent and receptor-dependent transmissions. (1) According to receptor-independent transmissions, CCK-8S may reach the brain by passive diffusion within minutes due to the bulk flow of cerebrospinal fluid along the olfactory or accessory olfactory nerves. Likewise, CCK-8S may passively diffuse into the cranial nerves and reach the brain by anterograde axonal transport (Balin et al., 1986; Bargman, 1996). (2) Receptor-dependent transmissions of intranasally administered CCK-8S into the brain may be due either to a receptor-mediated active transport into the olfactory or accessory olfactory nerves and a subsequent transport of

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the peptide into the brain. Alternatively, CCK-8S might bind to receptors located within the nasal cavity, presumably at the olfactory or accessory olfactory nerves, like the vomeronasal organ (VNO; Dudley et al., 1996; Kikuyama et al., 1995; Halpern, 1987), and induce a subsequent electrical transduction of this information into the brain.

The central nervous effects of CCK-8S can be reflected in event-related brain potentials (ERPs). ERPs are electrocortical potential changes, which can be measured before, during and after a sensoric, motoric or psychological event. They are regarded as electrophysiological brain correlates of a subject's stimulus processing. The psychological process of attentional stimulus processing appears to be mainly associated with the P300 (also termed late positive complex—LPC; Spencer et al., 2001; Näätänen, 1990). The LPC occurs within 300–800 ms after stimulus onset (Sutton et al., 1965). As cortical, thalamic and hippocampal or amygdala structures seem to contribute to the LPC generation, a parietal dominance is observed (Halgren et al., 1980). The LPC can be subdivided into two major positive components, which reflect different cognitive processes: the P3 (also termed P3b) and the subsequent slow wave (SW).

The P3 is a relatively large positive-going potential of the ERP, being elicited in normal young adults within a peak latency window of 250–450 ms (depending on stimulus, subject and environmental factors) after stimulus onset, with a posterior–parietal scalp distribution. It is associated with controlled (nonautomatic) stimulus processing (decoding of information) and task preparation (Sutton and Ruchkin, 1984; Polich and Kok, 1995; Kok, 2001). Also, it reflects fundamental cognitive processes related to memory access, memory load and allocation of attentional resources (Kok, 2001; Garcia-Larrea and Cezanne-Bert, 1998; Ravden and Polich, 1998; Humphreys and Kramer, 1994; Wickens et al., 1983). Accordingly, an increase in the P3 amplitude indicates a task-induced activation and maintenance of the working memory, if the internal model of the environment or context is updated and restructured by actual processing stimuli (Donchin, 1981; Donchin and Coles, 1988). Thus, the P3 (amplitude) may not only reflect part of an update between old and new representations of environmental stimuli, but may also represent the subsequent memory storage process to enable the subject to use current information in order to prepare for future events (Hoffmann, 1990). Moreover, P3 reflects a process of memory access necessary for the evaluation of stimuli in tasks that require decision and response actions (Kok, 2001).

An increase of the P3 may also indicate the allocation of attentional resources, as the P3 is proportional to the amount of attentional resources dedicated to a given task. Likewise, the P3 latency is correlated with mental functions, i.e., shorter latencies are associated with superior cognitive performance (Polich and Herbst, 2000). In particular, under high constraint conditions, such as a high working memory load, ERPs reveal a P3 positivity that increases in amplitude with memory load (Ruchkin et al., 1992).

Sometimes overlapping, the P3 is followed by the SW within a time domain of 400–700 ms after stimulus onset (Bradley, 2000). While the SW has a pronounced positivity at parietal recordings, it usually shows a sustained negativity at frontal recordings (Spencer et al., 2001). The SW occurs when the stimuli presented are relevant for resolving a demanding task. Cognitive capacity, effort and duration of encoding processes being necessary for the performance of an operation are represented in the magnitude of the SW (Ruchkin et al., 1992; Johnson and Donchin, 1985; Sutton and Ruchkin, 1984).

Few studies in humans have examined the effects of intranasally administered CCK on the brain. Pietrowsky et al. (1996) found effects of CCK-8S on auditory ERPs to be stronger after intranasal (in) than after intravenous (iv) administration, although plasma concentrations were comparable for both routes of administration. After intranasal administration of CCK-8S the increase of the LPC reached its maximum at 120 min after substance administration, the increase being more pronounced in women (Pietrowsky et al., 2001). In women only, the LPC already differed significantly from the placebo condition 30 min after administration, thus, indicating a possibly higher sensitivity of the female brain for CCK-8S. Both experiments used doses of 10 μg in CCK-8S.

The present study aimed at examining the effects of intranasally administered CCK-8S in two different single doses (10 and 20 μg) on central nervous processing as reflected by ERPs. The main question was, whether the dose of 20 μg CCK-8S would lead to stronger effects on the LPC and on behavioral indicators of controlled stimulus processing than 10 μg CCK-8S. The results should give further hints to the understanding of the yet unknown underlying mechanisms of the transmission from the nose to the brain. We hypothesized that, in case of a receptor-independent transmission of CCK-8S into the brain (e.g., due to the bulk flow of cerebrospinal fluid), the administration of a higher dose of CCK-8S should result in a more pronounced central nervous response, i.e., in a dose-dependent increase of the LPC. If, in contrast, the nose–brain pathway for CCK-8S relies on an active receptor-dependent transmission, larger doses are expected to exert saturation effects on ERP responses due to saturation of the receptor binding. Since previous studies revealed larger P300 effects of intranasal CCK-8S in women then in men, gender differences were additionally examined.

2. Method

2.1. Subjects

Subjects were 10 female and 10 male healthy, nonsmoking volunteer students without hearing loss aged 21–38 years (26.55 ± 1.09), who were paid for participation. In order to avoid endogenous CCK secretion, Ss were

instructed to fast overnight prior to each experimental session (i.e., to abstain from food or beverages except water or unsweetened herbal tea for at least 8 h). The experimental protocol was approved by the Ethics Committee of the Medical Faculty of Heinrich-Heine University, Duesseldorf, Germany, in compliance with the Declaration of Helsinki for human subjects. Subjects gave written informed consent.

2.2. Design and procedure

The study was conducted according to a double-blind within-subject cross-over design. With 7-day-intervals, each subject was tested on three consecutive sessions. At one session, subjects received either an intranasal administration of (1) a dose of 10 μg CCK-8S (Calbiochem-Novabiochem, Germany) or (2) a dose of 20 μg CCK-8S or (3) placebo. CCK-8S was dissolved in 4-ml sterile water. A dose of 5 μg CCK-8S (contained in one puff of 100 μl) was sprayed in each nostril. The placebo treatment consisted of sterile water, also administered as a puff of 100 μl in each nostril. In each condition, four puffs were administered. In the placebo condition, all puffs contained water. In the 10 μg CCK-8S condition, two puffs contained CCK, the other two water and, in the 20 μg CCK-8S condition, all four puffs contained CCK-8S. The selected initial dose of 10 μg CCK-8S based on results of previous studies and has been proven as sufficient for evoking LPC-modulations (cf. Pietrowsky et al., 1996, 2001). Sessions were always scheduled at 9 a.m. and 11 a.m. and lasted 1 h. The order of treatment was balanced according to a Latin square.

In order to ensure a similar physiological baseline for all subjects prior to treatment basal measures of heart rate, blood pressure and saliva cortisol levels were taken. Heart rate and blood pressure were recorded by a sphygmomanometer (OMRON, Germany, according to the Riva-Rocci method). For cortisol determination, saliva was sampled by a salivette (Sarstedt, Germany) and frozen immediately after collection for later assessment (delayed salivacortisol fluorescence immunoassay DELFIA, intraassay coefficient of variance $\approx$10%; Dressendörfer et al., 1992).

Experimental sessions took place in a sound attenuated and electrically shielded room with the subject sitting in a reclining chair. At the beginning of each session, prior to the preparation for EEG-recordings, substances were administered. Thirty minutes after substance administration, Ss performed an auditory oddball task. During this task, the EEG was recorded to assess the ERPs. Subjects attended to a sequence of a total of 400 tone pips in each testing occasion (60 ms duration, 64 dB SPL intensity) presented binaurally via headphones. In order to prevent the initial audio clicks when presenting the tone pips, a sinus wave was artificially generated with zero as starting point (equivalent to fade-in of 250 μs). Thus, the rise and fall times of the pips amounted to 250 and 266 μs, for the 1000 and 1064 Hz tone pips, respectively. The pips were either standard pips (80% occurrence, pitch 1000 Hz) or target pips (20% occurrence, pitch 1064 Hz) randomly interspersed among the standard pips. Interstimulus interval varied randomly between 1 and 3 s (average 2 s). Subjects were instructed to press a joystick as fast and as accurately as possible (with the thumb of the dominant hand) whenever a target pip occurred. Subjects were also instructed to keep their gaze on a fixation mark (circle; Ø 15 cm) located centrally in front of them in order to avoid eye blinks and body movements during task performance. Prior to the experimental block, subjects performed an exercise task with a shortened series of 40 tone pips in order to guarantee the tone differentiation. The stimulus characteristics of the oddball task were the same in all testing sessions.

Reaction time (time between target onset and joystick press response) and performance in a letter-cancellation test (d2) (Brickenkamp, 1967) were assessed as behavioral indicators of CCK-induced effects. The d2 test is a standardized paper–pencil test, which requires a high degree of sustained attention and concentration under high perceptual load.

2.3. Recordings of ERPs and apparatus

During subjects' performance on the oddball task EEG recordings (high pass filter: 0.05 Hz, low pass filter: 30 Hz, −12 dB/octave) were obtained from nonpolarizable electrodes (Ag/AgCl, Ø 16 mm; GVB Geiselberger, Germany) 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 artefacts, the vertical electrooculogram (VEOG) was recorded from electrodes above and below the left eye. EEG and EOG signals were amplified and digitized by a Synamps amplifier (Neuroscan, USA) at a rate of 1000 Hz. The unusual high digitization rate of 1000 Hz was due to an initial recording adjustment for further recordings. It was maintained for analysis. All data were stored on a computer disk for off-line averaging of ERPs.

2.4. Data reduction and analysis

Individual ERPs were averaged separately for each subject and each of the experimental conditions, which were: treatment (10 μg CCK-8S, 20 μg CCK-8S, placebo), type of tone pip (standard, target) and electrode site (Fz, Cz, Pz). The averaging period covered a 100 ms prestimulus baseline and a 800 ms poststimulus interval. Periods were excluded from analysis in case of eye blinks, gross eye movements or other potentials exceeding $\pm$75 μV.

N1 (between 60 and 130 ms poststimulus) and P2 (between 130 and 320 ms poststimulus) amplitudes and peak latencies were determined in the ERPs to the standard stimuli. N2 (between 160 and 320 ms poststimulus), LPC amplitudes and peak latencies were determined in the ERPs

to the target stimuli. Moreover, areas under the curve (AUC) were calculated for the total LPC and its subcomponents (P3, SW) in the ERPs to the target stimuli. The LPC was calculated between 280 and 700 ms poststimulus, the P3 between 280 and 500 ms poststimulus and the SW between 500 and 700 ms poststimulus.

Statistical evaluation of the ERP measures was based on repeated measures analyses of variance (ANOVA). ANOVA included a grouping factor for subjects' gender and repeated measures factors for the treatment conditions and electrode sites. In case of a significant treatment main effect, subsequent ANOVAs were run for each two treatment conditions to determine the source of this effect. Analyses were run separately for standard and target tone pips. Endocrine and cardiovascular measures as well as the detecting accuracy of target pips, mean reaction time and performance on the d2 letter-cancellation test were also based on repeated measures ANOVA with the treatment factor. A Greenhouse–Geisser corrected P value $\leq .05$ was considered significant. All values represent means ± S.E.M.

3. Results

3.1. Endocrine and cardiovascular measures

Cortisol levels prior to intranasal administration of CCK-8S showed neither significant differences between treatment conditions nor between men and women. Also, heart rate, systolic and diastolic blood pressure were within the normal range and not different between the treatment conditions.

3.2. Behavioral measures

Although the auditory oddball task appears to include a quite difficult discrimination (differentiation of tones bet-

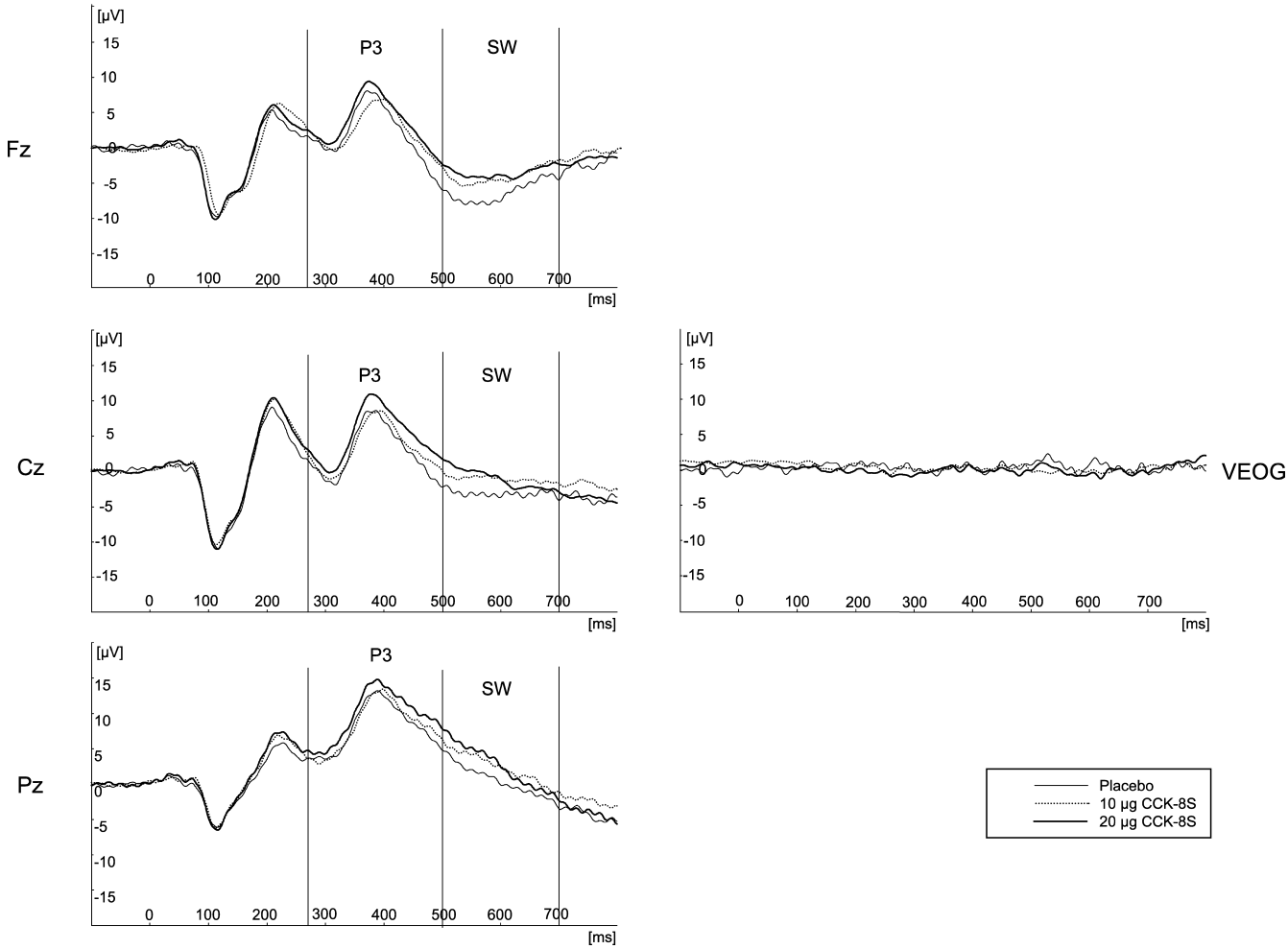

Fig. 1. Grand average ERPs recorded from three midline electrode sites (Fz, Cz and Pz) and the VEOG following the intranasal administration of placebo (thin solid line), 10 μg CCK-8S (dotted line) and 20 μg CCK-8S (thick solid line). Additionally, the separation of the LPC into a P3 subcomponent (280–500 ms) and a SW subcomponent (500–700 ms) is shown. Positivity is upward.

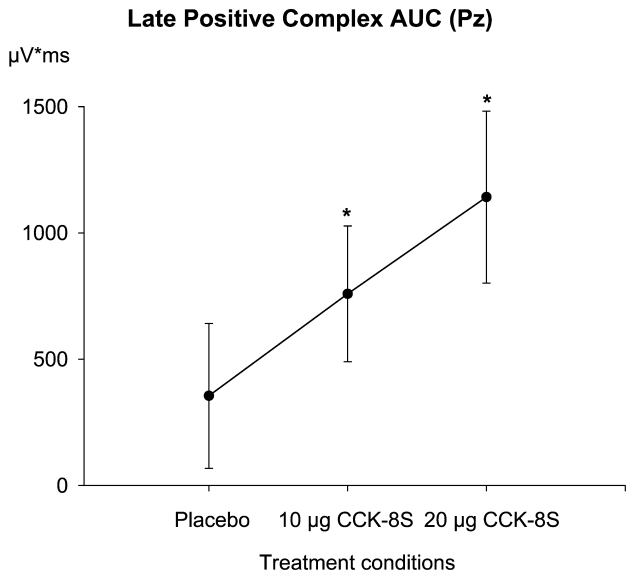

Fig. 2. Effects of intranasal application of placebo, 10 μg CCK–8S and 20 μg CCK-8S on the AUC of the LPC within the 280–700-ms poststimulus interval following target pips at parietal recordings (mean ± S.E.M., * $P < .05$ compared to placebo).

ween 1000 and 1064 Hz), subjects performed well though with significant differences in detecting the target pips (error rate for the placebo condition: 5.50%, for the 10 μg CCK-8S condition: 3.38% and for the 20 μg CCK-8S condition: 1.37%; $F(2,36) = 5.63$, $P < .05$). The error rate produced in the 10 μg CCK-8S condition as well as in the 20 μg CCK-8S condition was significantly lower compared to placebo [$t(19) = -2.62$, $P < .05$; $t(19) = -2.90$, $P < .05$, respectively].

The mean reaction time (placebo: 487.63 ± 10.80 ms, 10 μg CCK-8S: 494.44 ± 13.10 ms, 20 μg CCK-8S: 470.25 ± 13.06 ms; n.s.) until detection of targets in the oddball task as well as performance on the letter-cancellation test (d2) were not significantly affected by the kind of treatment.

3.3. ERPs

Fig. 1 shows the grand mean averages of the ERP recordings of the midline electrode sites (Fz, Cz, Pz) to the target stimuli for each of the three treatment conditions (placebo, 10 μg CCK-8S, 20 μg CCK-8S). The topographical distribution of the ERPs resembles the typical distribution observed in ERPs during performance on an oddball task, i.e., at Pz, the LPC is more pronounced than at frontocentral recording sites.

N1 (within 60–130 ms): the N1 amplitude was largest at Cz (−17.56 ± 0.92 μV) and smallest at Pz (−12.87 ± 0.65 μV; main effect electrode site: $F(2,36) = 41.43$, $P < .001$). No significant treatment effect was observed for N1 amplitude. N1 latency was not affected by any of the experimental variables.

P2 (within 130–220 ms): the P2 amplitude was also largest at Cz (12.23 ± 1.04 μV) and smallest at Pz (7.69 ± 0.87 μV; main effect electrode site: $F(2,36) = 48.73$, $P < .001$). No significant treatment effect was observed for P2 amplitude. P2 latency was shortest at Fz (209.14 ± 1.99 ms) and longest at Pz (212.25 ± 2.06 ms; main effect electrode site: $F(2,36) = 7.27$, $P < .01$).

N2 (within 160–320 ms): the N2 amplitude was largest at Fz (−5.00 ± 0.62 μV) and smallest at Pz (−1.57 ± 0.47 μV; main effect electrode site: $F(2,36) = 45.04$, $P < .001$). N2 amplitude was largest following placebo (−4.28 ± 0.66 μV) and smallest following 10 μg CCK-8S (−3.21 ± 0.56 μV; main effect treatment: $F(2,36) = 3.99$, $P < .05$). N2 latency was not affected by any of the experimental variables.

Table 1
Summary of the effects on the ERP components

ERP components	Effects	*F*	df	*P*	Comments
N1 amplitude	main effect electrode site	41.43	2,36	<.001	Cz>Fz>Pz
N1 latency	no effects				
P2 amplitude	main effect electrode site	48.73	2,36	<.001	Cz>Fz>Pz
P2 latency	main effect electrode site	7.27	2,36	<.01	Fz<Cz<Pz
N2 amplitude	main effect electrode site	45.04	2,36	<.001	Fz>Cz>Pz
	main effect treatment	3.99	2,36	<.05	placebo>20 μg>10 μg CCK-8S
N2 latency	no effects				
P3 amplitude	main effect electrode site	31.65	2,36	<.001	Pz>Cz>Fz
P3 latency	main effect electrode site	21.73	2,36	<.001	Fz<Cz<Pz
P3 AUC	main effect electrode site	62.81	2,36	<.001	Pz>Cz>Fz
SW AUC	main effect electrode site	34.08	2,36	<.001	Pz>Cz>Fz
	main effect treatment	5.54	2,36	<.01	20 μg>10 μg CCK-8S> placebo
LPC AUC	main effect electrode site	64.16	2,36	<.001	Pz>Cz>Fz
	main effect treatment	4.06	2,36	<.05	20 μg>10 μg CCK-8S> placebo

AUC = area under the curve.

P3 (within 280–500 ms): the P3 amplitude was largest at Pz (16.81 ± 1.41 μV) and smallest at Fz (11.39 ± 1.15 μV; main effect electrode site: $F(2,36) = 31.65$, $P < .001$). P3 AUC was likewise largest at Pz (1931.79 ± 249.82 μV*ms) and smallest at Fz (596.47 ± 173.64 μV*ms; main effect electrode site: $F(2,36) = 62.81$, $P < .001$). Neither P3 amplitude nor P3 AUC was significantly affected by the treatment conditions. P3 latency was shortest at Fz (352.72 ± 8.89 ms) and longest at Pz (382.97 ± 8.23 ms; main effect electrode site: $F(2,36) = 21.73$, $P < .001$).

SW (within 500–700 ms): the SW AUC was largest at Pz (356.83 ± 194.56 μV* ms) and smallest at Fz (−909.99 ± 139.01 μV* ms; main effect electrode site: $F(2,36) = 34.08$, $P < .001$). The SW also differed significantly between the treatment conditions (main effect treatment: $F(2,36) = 5.54$, $P < .01$). Single comparisons between each two treatment conditions showed an increased SW (positivity) following both doses of CCK-8S compared to placebo (10 μg: −168.24 ± 137.43 μV* ms; $F(1,18) = 7.28$, $P < .05$; 20 μg: −151.41 ± 183.67 μV* ms; $F(1,18) = 8.55$, $P < .01$; placebo: −590.05 ± 176.57 μV* ms. The negative SW values are caused by the ERP contributions from frontal and central electrode sites (slow frontal negative shift).

LPC (within 280–700 ms): the AUC of the total LPC was largest at Pz (2288.54 ± 360.27 μV* ms) and smallest at Fz (−313.53 ± 208.34 μV* ms; main effect electrode site: $F(2,36) = 64.16$, $P < .001$). Like for the SW subcomponent, the total LPC area also differed significantly between the treatment conditions (main effect treatment: $F(2,36) = 4.06$, $P < .05$). Single comparisons between each two treatment conditions revealed a larger LPC AUC for both doses of CCK-8S than for placebo (10 μg: 758.79 ± 268.62 μV* ms; $F(1,18) = 4.38$, $P < .05$; 20 μg: 1141.78 ± 340.49 μV* ms; $F(1,18) = 6.54$, $P < .05$; placebo: 354.87 ± 286.92 μV* ms; Fig. 2).

Table 1 presents a summary of the findings on the various ERP components. In general, there were neither significant effects of the subjects' gender on the ERP components nor significant Treatment × Electrode site interactions.

4. Discussion

The present study on dose–response relationships of intranasally administered CCK-8S on the P300 ERP indicates that both doses of the peptide substantially increased the LPC magnitude compared to placebo. This effect was more pronounced on the SW subcomponent of the LPC, while the P3 subcomponent of the LPC was nearly unaffected by the treatment. Moreover, CCK-8S also diminished the N2 amplitude. In line with the increased LPC, this result suggests that the peptide induced a positive shift of the ERP to target stimuli. The increase of the SW subcomponent of the LPC, which was repeatedly demonstrated for the 10 μg CCK-8S dosage (Pietrowsky et al., 1996, 2001), did not benefit from the higher dose of CCK administered. Although the 20 μg CCK-8S increased the LPC more than the 10 μg CCK-8S does, the difference did not reach statistical significance. Moreover, regarding the SW, where the peptide effect is more distinctive, no difference between the two dosages occurred. Thus, the present results give rise to the explanation that the (functional) transmission of CCK-8S from the nose into the brain relies on a saturable mechanism.

Since it is still unknown how the intranasally administered CCK-8S exerts its central effects, the results of the present study are discussed in the light of the two general transport mechanisms, a receptor-independent and a receptor-dependent transmission. While receptor-dependent transmissions are regarded to be saturable, receptor-independent transmissions are not. Thus, the present result of similar effects of the two doses of intranasally administered CCK-8S indicates that the substance exerts its central nervous effects via a receptor-bound transport mechanism from the nose into the brain. However, receptors being sensitive for CCK-8S and possibly existing in the nasal cavity have not yet been found neither in animals nor in humans. Binding of CCK-8S to receptors in the nasal cavity does further imply two basically different forms of transmission into the brain: (1) a transport of the peptide within afferent nerves into the brain or (2) the binding to receptors elicits electrical responses which are conducted to the brain.

On the other hand, instead of being localized in the nose, a saturable receptor-dependent mechanism may be located in the central nervous structures, which generate the LPC. In line with this argumentation, CCK-8S would bind to receptors located in the brain after reaching the CNS via a receptor-independent nonsaturable mechanism (e.g., by diffusion into the cerebral fluid along afferent nerves). Thus, the LPC increase-similarly sensitive to both dosages of CCK-8S—may be caused by a possible saturation (ceiling effect) of receptors in brain areas generating the late ERP positivity.

On the basis of the present results, it can not be decided on which stage of the transmission from the nose into the brain the saturable mechanism acts. However, referring to previous results of a fast action (within 30 min) of intranasally administered CCK-8S on the LPC (Pietrowsky et al., 2001), a transport of the peptide within afferent nerves is rather unlikely, because this way of transport takes several hours (Balin et al., 1986). Thus, there remain two principal forms of saturable transmissions of intranasally administered CCK-8S effects to the brain: (1) the peptide binds to receptors in the nasal cavity and elicits electrical responses, which then affect the brain structures that generate or modulate the LPC; (2) the peptide gets into the brain, e.g., by the bulk flow of cerebrospinal fluid along olfactory or accessory olfactory nerves, binding then to receptors within the CNS.

Like in previous studies with CCK effects on ERPs (e.g., Pietrowsky et al., 1996, 1997, 2001), these effects concentrated on the N2 and the LPC, i.e., indicators of controlled stimulus processing. ERP components reflecting automatic processing, like the N1 and P2, were not affected by the

peptide. In contrast to previous studies (Pietrowsky et al., 1997, 2001) however, the central nervous effects of CCK on ERPs were accompanied by respective changes in a behavioral measure. Although mean reaction time on the target pips and the performance on the letter-cancellation test were not affected by CCK-8S, the peptide improved the detection accuracy of the target tones in a dose-dependent manner. As indicated by the low error rates, the oddball task seemed to be of a minor difficulty. Thus, CCK-8S could possibly alleviate the maintenance of the tonic alertness over a longer time, as required by the task instruction, and further reduce the error rates. The improvement of the detection accuracy parallels the increase of ERP positivity by CCK and is in accordance with an improvement of controlled stimulus processing as indicated by an increase of the LPC (Hoffmann, 1990).

Although the CCK-induced increase of the LPC was more pronounced in women than in men, this effect did not reach significance. This result contrasts with previous studies, which demonstrated gender differences for the LPC increase following intranasal CCK-8S (Pietrowsky et al., 1996, 2001) with this effect being more distinctive in women than in men. A possible higher sensitivity of the female brain to CCK effects may result from interactions of CCK-8S with estrogen (Karlsson et al., 1992; Oro et al., 1988).

In sum, the present study demonstrates that a dose of 20 μg CCK-8S does not substantially alter the increase of the LPC compared to a dose of 10 μg CCK-8S. Thus, this cortical measure, which is regarded to be sensitive to CCK, does not benefit from increasing the dose administered. The failure of a further significant increase of the LPC by 20 μg CCK-8S compared to 10 μg CCK-8S may thus reflect a ceiling effect specific for this measure. Besides further evidence for possible mechanisms underlying the nose–brain pathway, the results also support the notion that CCK-8S improves controlled stimulus processing.

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