The Peptidergic Neuron
B. Krisch and R. Mentlein (eds)

Functional evidence for a transmission of peptides along the olfactory systems into the brain in healthy humans

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Summary. Some peptides are known to affect central nervous processing in humans after intravenous and intranasal application. These effects were assumed to be mediated via the peripheral blood. However, this assumption was challenged by the observation that for some peptides the intranasal application was more effective than the intravenous application in evoking central nervous effects. The present study served to test the hypothesis that following intranasal application, peptides exert central nervous effects that are not mediated by peripheral blood. In two experiments in healthy humans vasopressin (AVP) or cholecystokinin (CCK) were administered intranasally and intravenously in doses, comparable with respect to the induced plasma levels in the systemic blood. Cortical event-related potentials (ERPs) served as indicators of central nervous processing. The results indicate that under comparable plasma levels of vasopressin and cholecystokinin following intravenous or intransal administration, pronounced ERP-effects were observed only after intranasal, but not after intravenous application. It is concluded, that peptides can affect the brain via the olfactory or accessory olfactory systems.

Introduction

A number of peptides induce central nervous effects when administered intravenously or intranasally in humans. Following both routes of administration, the effects were assumed to be mediated via the peripheral blood. The peptides were assumed to exert central nervous effects by circumventing the blood-brain-barrier by several possible mechanisms, e.g. via the circumventricular organs or by acting on afferent nerves.

This view was challenged by two observations: first, in a review of human studies on behavioural effects of vasopressin the effects appeared to be stronger following intranasal than intravenous administration of the peptide (Fehm-Wolfsdorf and Born, 1991). Like intravenously administered peptides, intranasally administered peptides were assumed to

affect the brain after absorption into systemic blood. Since peptides do not penetrate the blood-brain barrier, several mechanisms were assumed mediating central nervous effects of blood-borne peptides, such as binding sites in the circumventricular organs and at peripheral nerve endings. Second, a number of substances has been shown to enter the brain via the intranasal route, like amino acids, viruses and heavy metals (Eseri and Tomlinson, 1984; Balin et al., 1986; Barnett and Perlman, 1993). Based on these observations, we supposed that peptides given intranasal also can directly affect the brain without prior entrance of the vascular system. To test this hypotheses two experiments were performed with vasopressin (AVP) and cholecystokinin (CCK). In pilot studies dosages for the intranasal and intravenous administration of the peptides were determined, resulting in comparable increases in the plasma level of the respective peptide. Central nervous effects were assessed by the recording of event-related potentials following administration of the peptides.

Materials and methods

The experiments were performed in young, healthy volunteer subjects (n = 15 in experiment 1, n = 20 in experiment 2) with a mean age of 24.1 years. They were designed as within-subject cross-over comparisons. The order of treatments was balanced according to a Latin square. In experiment 1, each subject received (1) intranasally a dose of 20 IU (43.50 ng/ml) vasopressin, (2) intravenously a dose of 0.025 IU (0.054 ng/ml) vasopressin, (3) intravenously a dose of 1.5 IU (3.26 ng/ml) vasopressin, and (4) placebo. In experiment 2, each subject received (1) intranasally a dose of 10 μg CCK, (2) intravenously a dose of 0.25 μg CCK, (3) intravenously a dose of 2.5 μg CCK, and (4) placebo. The respective half-lives are about 10 minutes for vasopressin and 3 minutes for CCK. To hold the subjects and the experimenter blind with respect to the experimental conditions, the subjects received additionally saline solution intravenously when the peptides were administered intranasally and saline solution intranasally when the peptides were given intravenously. The placebo treatment included intravenous and intranasal administrations

of saline solution. For the intravenous administration, vasopressin (Pitressin, Parke-Davis) or CCK-8 (Kinevac, Squibb) was dissolved in 100 ml saline solution and infused at a constant rate within 30 min. For intranasal administration, vasopressin (Bachem) or CCK-8 (Sigma) were dissolved in 10 ml sterile water and the respective dosages were administered as nasal spray (10 IU vasopressin per puff, 5 μg CCK per puff).

Recordings of event-related potentials started 45 min after treatment administration had been started and lasted for about 45 min (including a break of 10 min). Event-related-potentials were obtained while the subject performed on an auditory oddball task. In this task, sequences of tone pips were presented binaurally via headphones to the subjects. A sequence consisted of two types of pips: standard pips (90% probability, pitch: 800 Hz), and rare target pips (10% probability, pitch 840 Hz) which were randomly interspersed among the frequent standard pips. Interstimulus intervals varied randomly between 1 and 3 s. Subjects were instructed to react as fast and as accurately as possible with a button press response to target pips. In each session, the subject was presented with two sequences of tone pips each consisting of about 500 pips. To determine plasma peptide concentrations, blood samples were collected immediately prior to administration of treatments, and 10, 20, 30, 45, 70 and 100 min after the start of the vasopressin administration and 10, 20, and 30 min after the start of the CCK administration. Blood samples were immediately centrifuged and the plasma frozen at -20° C for later determination of plasma vasopressin and plasma CCK concentrations by radioimmunoassay. During the performance on the oddball task, recordings were obtained from electrodes attached according to the 10-20-system along the midline at Fz (frontal), Cz (central), and Pz (parietal).

Individual event-related-potentials were averaged separately for each subject and experimental condition which were: "Treatment", "Type of tone pip", and "Electrode site". The averaging epoch covered a 200 ms pre-stimulus baseline and an 800 ms post-stimulus interval. Peak amplitude and area under the curve was determined for the P3 component within 280-700 ms post-stimulus.

Results

In experiment 1, the intravenous administration of 1.5 IU vasopressin significantly increased plasma vasopressin levels compared to placebo, 0.025 IU vasopressin intravenous, and 20 IU vasopressin intranasal ($p<0.01$, respectively). Plasma vasopressin levels following 0.025 IU vasopressin intravenous and following 20 IU vasopressin intranasal were nearly identical (Fig. 1). The change in P3 amplitude was not correlated with the increase in plasma vasopressin. P3 amplitude was significantly increased following administration of 20 IU vasopressin intranasally compared to the effects of all other treatments, i.e., to the administration of 1.5 IU vasopressin intravenously ($p<0.05$), of 0.025 IU vasopressin intravenously ($p<0.05$) and of placebo ($p<0.05$; Fig. 1).

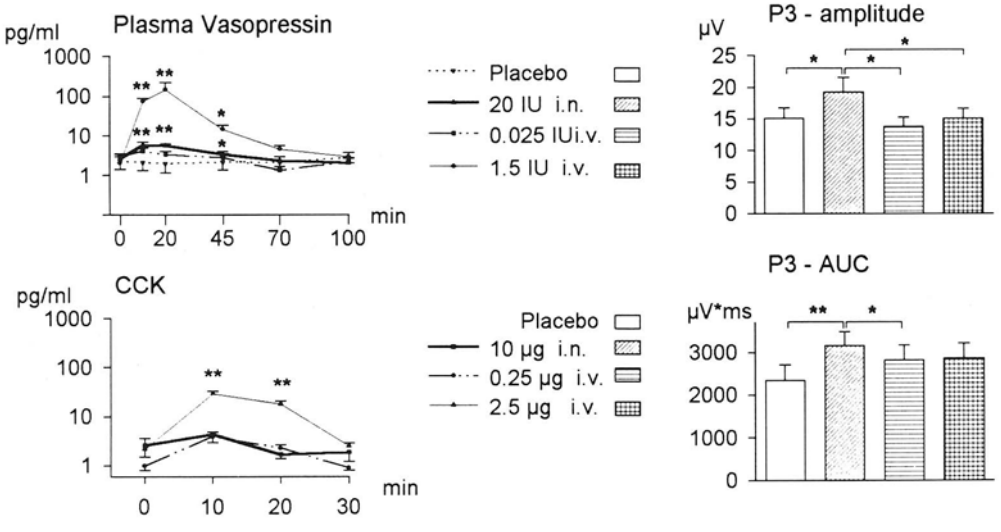

Fig. 1. Top left: Plasma vasopressin (AVP) levels following 20 IU AVP intranasal (i.n.), 0.025 IU AVP intravenous (i.v.), 1.5 IU AVP i.v., and placebo at 0, 10, 20, 30, 45, 70, and 100 min following administration. Right: Peak amplitude of the P3 component following placebo and i.n. and i.v. AVP administrations. **Bottom** left: Plasma CCK levels following 10 µg CCK i.n., 0.25 µg CCK i.v., and 2.5 µg CCK i.v. Right: Area under the curve (AUC) of the P3 amplitude following placebo and i.n. and i.v. CCK-8 administration, * $p<0.05$, ** $p<0.01$.

In experiment 2, CCK plasma concentrations were distinctly higher following the 2.5 μg CCK intravenous administration compared to the other treatments ($p<0.01$). The plasma CCK concentrations following intranasal administration of 10 μg CCK and following intravenous administration of 0.25 μg CCK were nearly identical (Fig. 1). P3 area under the curve was significantly increased following the intranasal administration of CCK compared to placebo ($p<0.01$) and to the low dose (0.25 μg) intravenous CCK ($p<0.05$; Fig. 1).

Discussion

The results show central nervous effects of peptides administered intranasally or intravenously in doses leading to comparable plasma levels, selectively following the intranasal route of administration. This excludes that the central nervous effects of these peptides when given intranasally emerged after entrance of the peptide into systemic blood. Results suggest a direct pathway from the nose to the brain for peptides, as was previously shown for other substances (e.g. Balin et al., 1986; Barthold, 1988; Barnett and Perlman, 1993).

While the lower dose of peptides administered intravenously (with similar plasma levels to the intranasal administration) did not affect event-related potentials, moderate effects were observed after high doses of intravenously admininistered CCK. This observation indicates that peptides circulating in the peripheral blood are not completely ineffective with regard to central nervous actions. However, the mechanisms transferring effects of blood borne peptides across the blood-brain barrier on the brain are obviously different than those mediating brain effects of intranasally administered peptides.

Several mechanisms have been considered transporting molecules from the nose to the brain. Peptides could be taken up into olfactory nerves and transported intracellularly (Barthold, 1988; Perlman et al., 1990). Also, they may pass through intercellular clefts in the olfactory epithelium to diffuse extracellularly to further distant sites of the central nervous system expressing peptide receptors (Balin et al., 1986). Finally, substances may

influence the central nervous system not only by a spread along the olfactory system, but also along the accessory olfactory system, i.e., the vomeronasal organ, which is a chemosensory organ differing in morphology and neuroanatomical connections from the olfacory system (Monti-Bloch et al., 1994; Meredith and Fernandez- Fewell, 1994).

Conclusions

The present findings suggest that a direct nose-brain pathway exists for peptides. However, the results provide only functional evidence. Thus, it can not be decided whether peptides are transported into the brain following intranasal administration or whether their actions result from binding to receptors at peripheral sites of the olfactory systems. Nevertheless, since the actions of intranasally administered peptides do not depend on resorption into systemic blood, the nose-brain pathway may be of relevance for a more direct treatment of central nervous system diseases.

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