Brain Potential Changes after Intranasal vs. Intravenous Administration of Vasopressin: Evidence for a Direct Nose-Brain Pathway for Peptide Effects in Humans

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There is evidence that intranasal application of peptides is a way to circumvent the blood-brain barrier. This led us to compare the effects of arginine-vasopressin (AVP) on event-related potentials (ERPs) in healthy men ($n = 15$) *after intranasal and after intravenous (IV) administration. In a double-blind, crossover study, subjects received on three different occasions 20 IU of AVP intranasally (IN), 1.5 IU of AVP IV, and saline solution. ERPs were recorded during the subject's performance on an auditory attention task. Plasma concentrations of vasopressin during task performance were enhanced after AVP, with the increase after IV administration of AVP exceeding that after intranasal AVP* ($p < 0.05$). *Intranasal administration of AVP substantially increased the P3 component of the ERP* ($p < 0.01$). *By contrast, IV administration of AVP had no consistent effects on the ERP responses. In supplementary experiments as well, IV administration of lower doses of AVP (0.1 and 0.025 IU) did not affect the ERP. Plasma vasopressin concentrations after the 0.025 IU dose in these experiments were comparable to those after intranasal administration of 20 IU AVP. The results provide functional evidence that in the human brain effects of peptides like AVP may be facilitated after IN as compared to IV administration.*

Key Words: Arginine-vasopressin, event-related potentials (ERPs), P3, nose-brain pathway, human

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Introduction

A basic problem of human neuropharmacology is that a large number of substances with potential influences on central nervous neurotransmission do not enter the brain after systemic administration due to the presence of brain barriers. Various pathways through which normal brain barriers may be circumvented have been demonstrated in animal studies. Balin et al (1986) have provided anatomical evidence that, in rats and primates, large tracer molecules, such as native horseradish peroxidase, after intranasal administration, pass freely through intercellular junctions of the olfactory epithelia to reach the olfactory

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bulbs of the central nervous system (CNS) extracellularly within 45–90 min. Their results suggest that the brain uptake of these molecules is facilitated after intranasal (IN) administration as compared to the intravenous (IV) route of administration. Other possible mechanisms of a nose-brain signal transfer are the intraneuronal transport of substances or the degradation of substances in the nasal epithelium and a passing of centrally active fragments intra- or extraneuronally to the brain (Dahl et al 1991; Burbach et al 1993).

A number of experiments have shown that the peptide vasopressin (and related substances) affects brain functions after IN administration in humans. Arginine-vasopressin (AVP) increased the amplitude of several components of the late event-related potential (ERPs), including the N1, P2, N2, and P3 (Fehm-Wolfsdorf et al 1984, 1988, Born et al 1986, 1987; Pietrowsky et al 1989; Naumann et al 1991; for a review, see Fehm-Wolfsdorf and Born 1991). A most pronounced increase in amplitude after IN administration of AVP has been found for the P3 component (Naumann et al 1991).

The ERP represents a series of brain electrical responses to a stimulus, with the potential components of the ERP referring to specific stages of stimulus processing. Thus, each incoming stimulus in the auditory modality rather invariably elicits (around 100 msec poststimulus) the N1-P2 component complex, reflecting a nonspecific cortical arousal reaction presumably mediated via collateral activation of the reticular formation (Näätänen and Picton 1987). By contrast, the P3 component of the ERP (about 350 msec poststimulus) is selectively elicited by task-relevant stimuli to which the subject is required to respond (e.g., by pressing a button). Psychophysiologically, P3 amplitude has been considered to indicate context updating within working memory after processing a stimulus. Moreover, this context updating is subject to attentional requirements of the task, with P3 changing depending on attention allocation (Donchin and Coles 1988; Verleger 1988). N1, P2, and P3 are reliably recorded in an oddball task. This task consists of a randomized sequence of standard stimuli and targets, and the subject is instructed to respond selectively to the targets. In numerous studies, late ERP components, such as the N1, P2, and P3, have been proved a sensitive tool for the examination of pharmacologic and hormonal influences on human CNS functions (e.g., Born and Fehm 1988; Johnston and Wang 1991; Klorman and Brumaghim 1991).

The present experiments compared the effects of IN vs. IV administration of AVP on human brain functions. It was hypothesized that after intranasal administration, access of AVP to the brain is facilitated and may circumvent the blood compartment. Therefore, effects of AVP on ERPs were expected to be more pronounced after IN than IV administration, even if blood concentrations after IN administration are equal to or lower than concentrations after IV administration of the peptide. In the experiments, plasma vasopressin concentrations were monitored, and blood pressure was recorded to control for possible peripheral cardiovascular effects. As an assay for effects on the brain's stimulus processing, ERPs were recorded during the subject's performance on an oddball task.

Methods

Subjects

Experiments were conducted in 15 healthy male students aged 20–35 years (mean age 28.3 years) who were paid for participation. They were nonsmokers and had to abstain from alcohol, caffeine, and food intake for at least 12 hr prior to testing. They were required to eat a normal "German" dinner on the evening before the experimental session. Subjects were free of medication at the time of the experiments. The night before testing they had slept normally. Audiometric examination excluded any hearing deficits. 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

Each of 15 men participated in three test sessions after having received: (1) an intranasal dose of 20 IU AVP, (2) an intravenous dose of 1.5 IU AVP, and (3) placebo. To blind the subject and the experimenter, the subject also received saline solution IV when AVP was administered IN. When AVP was given IV, saline solution was given, in addition, IN. The placebo treatment consisted of IV and IN administration of saline solution. Intranasal administrations took place at 9:00 AM; at that time, IV infusions were also started. Experiments were designed according to a within-subject crossover design. The order of treatments was balanced according to a Latin square, i.e., 5 subjects received IN AVP on their first testing occasion, 5 other subjects received AVP IV first, and the remaining 5 subjects received placebo first. Experimental sessions for a subject were at least 1 week apart.

For IV infusion, AVP (Pitressin, Parke-Davis, Germany) was dissolved in 100 mL saline solution and administered as constant rate infusion within 20 min. For IN administration, AVP (Bachem, Switzerland) was dissolved in 10 μl sterile water and a dose of 10 IU was blown in each nostril with a Rhinüle (Ferring, Germany). The Rhinüle is a small, flexible tube with a tip on one end

that allows one to blow a defined volume of 0.2 mL of a liquid substance into one's nostril.

Experiments were conducted in a sound-attenuated and electrically shielded room, with the subject sitting in a reclining chair. Recording of ERPs started 45 min after treatment administration had been started (i.e., 9:45 AM) and lasted for about 45 min (including a break of 10 min). ERPs were obtained while the subject performed an auditory oddball task. On this task, sequences of tone pips (duration: 60 msec, rise/fall time: 5 msec, intensity: 56 dB SPL) were presented binaurally via headphones to the subjects. A sequence consisted of two types of pips: standard pips (90% probable, pitch: 800 Hz) and rare target pips (10% probable, pitch: 840 Hz), which were randomly interspersed among the frequent standard pips. Interstimulus intervals varied randomly between 1 and 3 sec (average 2 sec). Subjects were instructed to react as fast and as accurately as possible with a button press response to target pips (with the thumb of the dominant hand). 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. In each session, the subject was presented with two sequences of tone pips, each consisting of about 500 pips. The sequences were separated by a resting interval of about 10 min.

For blood sampling and IV administration of substances, a catheter was placed in the vena cephalica of each arm. To determine plasma vasopressin concentrations, blood samples were collected immediately prior to administration of treatments and 10, 20, 45, 70, and 100 min after the start of treatment. Samples were collected 45, 70, and 100 min following administration of the substance, immediately before presentation of the oddball task, within the break between the two sequences of the task, and after task performance. Blood pressure was measured prior to the application of substances, prior to task performance, between the two sequences of the oddball task, and after task performance.

Recordings

During the subject's performance on the oddball task, EEG recordings (high-pass filter: 0.03 Hz −3 dB, −6 dB/octave; low-pass filter: 70 Hz, −3 dB, −12 dB/octave) were obtained from nonpolarizable electrodes (Ag/AgCl, diameter: 16 mm, Beckman Instruments, USA) attached along the midline at Fz, Cz, and Pz. Linked electrodes attached to the mastoids of the right and left ear served as reference. The ground electrode was attached to the forehead. For artifact detection, the vertical electrooculogram (EOG) was recorded by electrodes above and below the left eye. EEG and EOG signals were amplified by a Nicolet EEG 1A97 amplifier and digitized (CED 1401, Cambridge Electronic Design, UK) at a sampling rate of 200 Hz for off-line averaging of ERPs.

Blood pressure was measured automatically by a BC 40 (Bosch und Sohn, FRG), simulating the Riva-Rocci procedure. Blood samples were immediately centrifuged and the plasma frozen at −20°C for later determination of plasma vasopressin concentrations by radioimmunoassay (Hermann Biermann, FRG; sensitivity: 0.6 pg/mL, intra-assay coefficient of variation: < 10% between 0.6 and 30.0 pg/mL). Samples from an individual subject were analyzed in duplicate in the same assay. AVP measures of one subject could not be determined due to a technical failure.

Data Reduction and Analysis

Individual ERPs were averaged separately for each subject and experimental condition, which were: treatment (placebo, IN AVP, IV AVP), type of tone pip (standard, target), and electrode site (Fz, Cz, Pz). ERPs were collapsed across the two task sequences (first and second). The averaging epoch covered a 200 msec prestimulus baseline and an 800 msec poststimulus interval. Epochs were excluded from analysis if they contained blinks, gross eye movements, or other potentials exceeding 150 μV. The mean rejection rate of sweeps due to artifacts was: placebo—standards, 5.2%; targets, 8.9%; IN AVP—standards, 5.6%; targets, 8.3%; IV AVP—standards, 4.9%; and targets, 8.8%.

Peak amplitudes (with reference to the average potential during the prestimulus baseline) were determined for ERP components within latency bins accounting for the N1 (maximum negativity 70–140 msec poststimulus), P2 (maximum positivity 130–230 msec), and P3 (280–600 msec). In addition, peak-to-peak amplitudes were calculated for the N1-P2 amplitude difference.

Statistical evaluation of the behavioral data, ERP measures, plasma AVP levels, and blood pressure was based on analyses of variance (ANOVAs) and multivariate analyses of variance (MANOVAs) using the BMD-P4V program. ANOVAs on behavioral data included a repeated measures factor for the treatment conditions (placebo, IN AVP, IV AVP). ANOVAs for plasma AVP concentration and blood pressure measures included repeated measures factors for treatment conditions and time of recording. MANOVAs on ERP measures included repeated measures factors for the treatment conditions and type of tone pip (standard, target), and electrode locations (Fz, Cz, Pz) as variates. For all parameters, first an overall analysis on all three treatment conditions was run. Subsequently, sub-ANOVAs between any two of the treatment conditions were computed. Furthermore, orthogonal contrasts were

computed for each time of recording (for plasma AVP concentrations) and for each electrode location (for ERP measures) within the MANOVA approach. This testing compensated for an increased probability of type I errors. P3 measures following target pips were statistically analyzed only for tone pips correctly discriminated (hits). A Greenhouse-Geisser corrected p-value < 0.05 was considered significant.

Results

Behavioral Data

Reaction time (RT) and error rate (ER) did not differ significantly among the three treatment groups (RT: placebo—441.2 ± 25.3 msec (mean ± SEM), IN AVP—429.8 ± 26.7 msec, IV AVP—436.4 ± 25.3 msec. ER hits: placebo—6.5% ± 4.0%, IN AVP—4.9% ± 4.2%, IV AVP—5.8% ± 2.4%). Although the tone discrimination was quite difficult, the error rate was relatively small (< 7%), i.e., more than 93% of the targets were correctly discriminated (hits) on all testing occasions.

ERPs

N1 and P2 after standard stimuli and after target stimuli were highest at Cz ($F(2,28) = 16.5, p < 0.001$ and $F(2,28) = 31.0, p < 0.001$, respectively) but were not affected by AVP treatment. There were also no substantial treatment effects on latencies of these components.

Although the auditory evoked P3 in an oddball task that is not easily identifiable after standard pips, an exploratory analysis was performed on P3 after these standard pips using a latency window between 280 and 600 msec poststimulus. P3 amplitude and latency measures derived from this procedure following standard pips did not depend on the treatments administered.

The P3 component elicited by target tones displayed its maximum positive amplitude at Pz ($F(2,28) = 37.2, p < 0.001$, Figures 1 and 2). P3 was enhanced after IN AVP across electrodes ($F(2,28) = 6.2, p < 0.01$). This enhancement reached 32% compared to placebo ($F(1,14) = 5.2$, $p < 0.05$ in a subsequent pairwise comparison to placebo). After IV administration of AVP, P3 amplitudes were comparable with those after placebo. Consequently, P3 was also significantly enhanced after IN AVP, when compared to effects of IV AVP ($F(1,14) = 11.0, p < 0.01$; Figure 2). The selective increase in P3 after IN administration of AVP (versus placebo and IV AVP) was also significant when comparisons were performed separately for recordings from each electrode site (Table 1). The electrode site × treatment interaction did not reach significance (4,56) = 0.8, NS). P3 latency did not differ among treatment conditions.

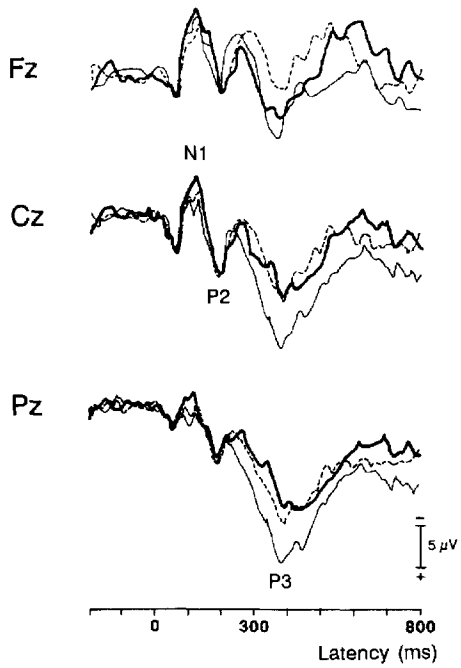

Figure 1. Averaged auditory evoked potentials to target stimuli of an attended oddball task from a single subject. Responses are plotted separately for the three treatment conditions: placebo *(thick solid line)*, intranasal administration of arginine-vasopressin (AVP) 20 IU *(thin solid line)*, and intravenous administration of AVP *(dashed line)*. Recordings were from Fz, Cz, and Pz (against linked mastoid references, vertex negative upward). N1, P2, and P3 components are marked for the electrode site of their respective maximum amplitudes.

Cardiovascular Parameters

Neither intranasal nor intravenous administration of AVP exerted any influence on systolic blood pressure. There was a slight decrease in diastolic blood pressure after intranasal administration of AVP, which—compared to the placebo condition—reached significance between the first and second sequence of ERP recordings (Table 2).

Plasma Vasopressin Concentrations

Plasma vasopressin concentrations were significantly increased between 10 and 45 min following IN administration of AVP, compared to placebo. Concentrations determined between the sequences of the oddball task, and after performance on this task, did not differ after intranasal administration of AVP and placebo (Figure 3).

Following IV administration of AVP, plasma vasopressin concentrations strongly increased; this increase substantially exceeded that after IN administration of AVP (Figure 3). Concentrations after IV administration were still above those after IN AVP immediately prior to ERP recordings (45 min) and between the two sequences of ERP recordings (70 min).

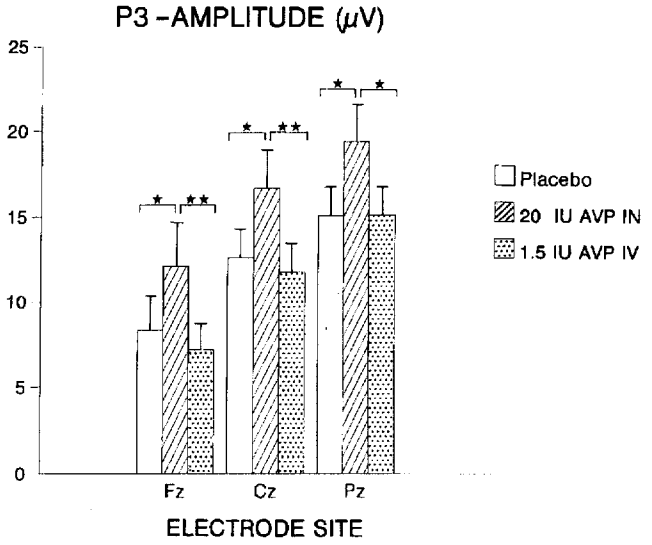

Figure 2. Mean (±SEM) peak-to-baseline amplitude (μV) of P3 after target stimuli of an auditory oddball task. Recordings from Fz, Cz, and Pz were obtained after administration of placebo *(white columns)*, after intranasal administration of arginine-vasopressin (AVP) 20 IU *(hatched columns)*, and after intravenous administration of AVP 1.5 IU *(dotted columns)*. $*p < 0.05$, $**p < 0.01$ for pairwise comparisons between any two of the treatment conditions.

To exclude that differential influences of IN versus IV administration of vasopressin on ERPs resulted from different vasopressin concentrations reached after the two routes of administration during and prior to ERP recordings, supplementary analyses and experiments were performed.

Supplementary Analyses and Experiments

Eight subjects were selected from the original sample of 15 subjects who showed comparable plasma vasopressin concentrations for the three samples collected prior to ERP recordings, between the sequences of the oddball task, and after task performance. Plasma vasopressin concentrations in this subsample for the different treatment conditions are shown in Table 2. In these subjects, too, P3 amplitude was markedly higher after IN administration of AVP (15.6 ± 4.0 μV) than after placebo (11.3 ± 2.4 μV) and after IV administration of AVP (9.4 ± 3.2 μV, $F(1,7) = 6.7$, $p < 0.05$). There were no effects of AVP on blood pressure in this subsample.

Given that peak plasma concentrations of vasopressin were significantly higher after intravenous than intranasal administration of AVP, it remained conceivable that the selective ERP effects of AVP after IV administration were due to an inverted *U*-shaped function of the relation between plasma AVP concentrations and ERP changes. Therefore, subsidiary experiments were run in 14 male students receiving IV doses of AVP lower than that in the main experiment, i.e.: (1) 0.1 IU AVP, (2) 0.025 IU AVP, and (3) saline solution as a control. Timing of infusions, blood collection, and ERP recordings were the same as in the main experiments. Plasma vasopressin concentrations after 0.025 IU AVP were comparable to those obtained

Table 1. ERP Amplitudes: Means (±SEM) of N1-P2 Difference Amplitude and of P3 Peak-to-Baseline Amplitude (in μV)

	Placebo (Pl)	AVP Intranasal (IN)	AVP Intravenous (IV)	Significance for Pairwise Comparisons
N1-P2[a]				
Standard tones				
Fz	10.8 (1.3)	11.6 (1.9)	10.5 (1.1)	NS
Cz	12.1 (1.2)	13.3 (1.9)	12.2 (1.0)	NS
Pz	7.9 (0.9)	8.4 (1.0)	8.4 (0.7)	NS
Target tones				
Fz	11.7 (1.2)	12.7 (1.4)	11.6 (0.9)	NS
Cz	12.6 (1.1)	14.3 (1.7)	12.9 (0.9)	NS
Pz	8.5 (1.1)	9.7 (1.1)	9.4 (0.9)	NS
P3[b]				
target tones				
Fz	8.4 (2.0)	12.2 (2.4)	7.2 (1.5)	Pl-IN[c], IN-IV[d]
Cz	12.7 (1.7)	16.6 (2.3)	11.8 (1.6)	Pl-IN[c], IN-IV[d]
Pz	15.1 (1.7)	19.3 (2.3)	15.1 (1.6)	Pl-IN[c], IN-IV[d]

[a]N1-P2 difference amplitude was determined for ERPs to standard and target pips of an oddball task.
[b]P3 was determined for ERPs to target pips of this task.
[c]$p < 0.05$
[d]$p < 0.01$.
ERPs were recorded after placebo, following IN administration of AVP (20 IU), and following IV administration of AVP (1.5 IU) from Fz, Cz, and Pz electrode sites.
ERP = event-related potential; AVP = arginine-vasopressin; NS = not significant.

Table 2. Mean (±SEM) Plasma Vasopressin Levels and Blood Pressure prior to ERP Recordings (pre), between the Two Sequences of ERP Recordings and after ERP Recordings (Post)

	Placebo (Pl)	AVP Intranasal (IN)	AVP Intravenous (IV)	Significance for Pairwise Comparisons
Total sample (n = 14)				
Plasma vasopressin				
pre	2.18 (0.8)	3.35 (0.7)	14.86 (4.4)	Pl-IN[a], Pl-IV[a], IN-IV[a]
between sequences	2.15 (0.8)	2.36 (0.6)	4.72 (1.0)	Pl-IV[b], IN-IV[a]
post	2.84 (0.8)	2.02 (0.7)	2.96 (0.9)	NS
Systolic blood pressure				
pre	110.3 (3.1)	109.4 (2.8)	112.5 (2.5)	NS
between sequences	111.6 (4.3)	106.6 (3.4)	109.1 (3.2)	NS
post	114.1 (3.2)	110.3 (3.4)	112.5 (3.0)	NS
Diastolic blood pressure				
pre	71.6 (2.4)	69.7 (1.5)	74.1 (1.5)	IN-IV[a]
between sequences	72.2 (1.7)	68.8 (1.7)	72.8 (1.8)	Pl-IN
post	75.9 (2.4)	72.5 (1.8)	74.4 (1.6)	NS
Subsample (n = 8)[c]				
Plasma vasopressin				
pre	2.72 (1.4)	4.29 (1.1)	8.15 (2.2)	Pl-IV[a]
between sequences	2.42 (1.3)	3.27 (1.0)	4.20 (1.4)	NS
post	3.54 (1.3)	2.53 (1.1)	3.05 (1.3)	NS
Systolic blood pressure				
pre	112.9 (4.1)	114.4 (3.4)	112.9 (1.8)	NS
between sequences	114.3 (3.7)	110.0 (3.8)	105.7 (4.8)	NS
post	112.9 (3.8)	109.3 (5.2)	110.0 (3.1)	NS
Diastolic blood pressure				
pre	73.5 (3.4)	70.7 (2.8)	72.9 (1.8)	NS
between sequences	75.7 (2.6)	72.1 (2.6)	70.0 (3.1)	NS
post	76.4 (4.2)	73.6 (2.4)	72.9 (1.7)	NS

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] Subsample of eight subjects for whom differences in vasopressin concentration after intranasal and intravenous administration of AVP during ERP recordings remained nonsignificant.
ERP = event-related potential; AVP = arginine-vasopressin; NS = not significant.

after IN administration of 20 IU AVP in the main experiments (Figure 3). Neither the 0.1 IU nor the 0.025 IU dose of AVP administered IV exerted any significant effect on ERPs. P3 amplitudes at Pz were 12.7 ± 1.8 μV after 0.1 IU AVP, 13.9 ± 1.4 μV after 0.025 IU AVP, and 13.0 ± 2.1 μV after saline solution. Also, blood pressure remained unaffected after IV administration of AVP in these experiments.

Discussion

The present experiments aimed to test central nervous effects of AVP depending on the route of administration. Intranasal administration of 20 IU AVP markedly increased the amplitude of the P3 component of the ERP recorded during the subject's performance on an oddball task. By contrast, the intravenous administration of AVP at various doses—although leading to higher plasma AVP levels in some cases—remained ineffective.

The present finding of an enhanced P3 after IN administration of AVP adds to previous results (Naumann et al 1991), which reported that an increased P3 was elicited by visually presented words after 30 IU AVP and 60 IU AVP were administered IN 48, 24, and 1 hr prior to testing. Effects of AVP in that study concentrated on frontocentral recordings. This is consistent with the present findings indicating that the P3 enhancement after intranasal AVP was not restricted to parietal electrode sites (where P3 is most prominent) but was also of comparable strength over frontocentral cortical areas. Considering that P3 is viewed as a manifestation of context updating in immediate memory, modified by the attention allocation requirements of the task, the P3 enhancement after intranasal vasopressin could well reflect an increase in attentional abilities; however, decreases in reaction times and error rate were too small and insignificant to substantiate this hypothesis on a behavioral level. Nevertheless, the view of an improving effect of AVP on attentional abilities is further supported by results from foregoing studies indicating that—depending on the task—AVP may also

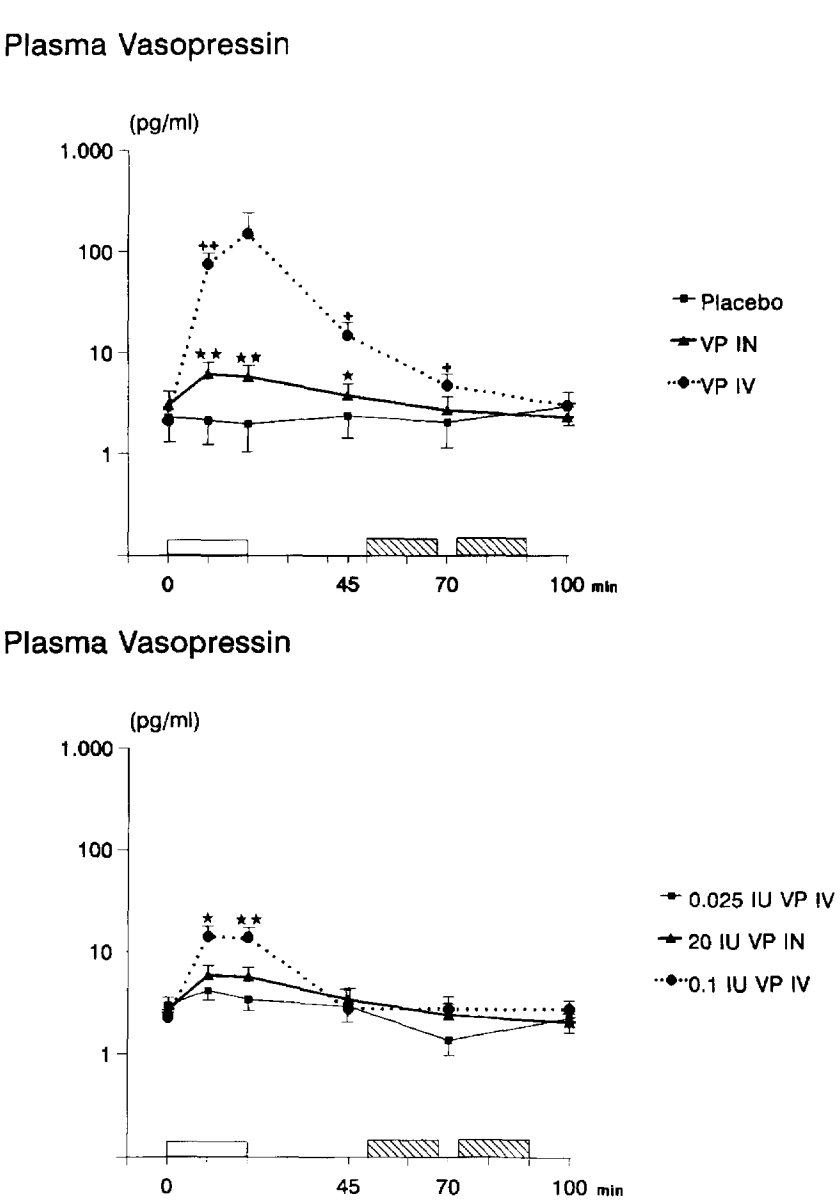

Figure 3. *Top:* Mean (±SEM) plasma vasopressin levels following administration of placebo *(thin solid line)*, intranasal (IN) administration of arginine-vasopressin (AVP) 20 IU *(thick solid line)*, and following intravenous (IV) administration of AVP 1.5 IU *(dotted line)*. Concentrations were determined immediately prior to treatment administration (0 min), and 10, 20, 45, 70, and 100 min later. Intravenously, AVP was infused within 20 min *(white bar)*. *Hatched bars* indicate time of ERP recordings. $*p < 0.05$, $**p < 0.01$, for comparisons with placebo; $+p < 0.05$, $++p < 0.01$, for comparisons between IN vs. IV administration of AVP. Note logarithmic scale of ordinate.
Bottom: Mean (±SEM) plasma vasopressin concentrations following IV administration of 0.1 IU AVP *(dotted line)* and 0.025 IU AVP *(thin solid line)*. Vasopressin concentrations in these conditions were compared with those obtained after IN administration of 20 IU AVP in the main experiment *(thick solid line)*. $*p < 0.05$, $**p < 0.01$, for comparisons with concentrations after IN administration of 20 IU AVP.

enhance the amplitude of late ERP components other than P3 (Timsit-Berthier et al 1982; Born et al 1986; Fehm-Wolfsdorf et al 1988; Pietrowsky et al 1989).

The main finding of the present experiment is the selective CNS influence of AVP given intranasally, whereas the intravenous administration of the peptide remained ineffective. Interestingly, a review of the literature on the effects of AVP (and related substances) on human brain functioning indicates that the majority of studies reporting on systematic changes after AVP used the IN route of administration (Timsit-Berthier et al 1982; Fehm-Wolfsdorf et al 1984, 1988; Born et al 1986, 1987; Pietrowsky et al 1989; Naumann et al 1991).

It is unlikely that the differences in CNS influences of AVP after IN vs. IV administration were due to diverging peripheral actions. Cardiovascular functions—considered a possible mediator of CNS effects of AVP (Le Moal et al 1981; Sahgal 1984)—were not systematically changed by AVP (at the administered doses). Although a slight but inconsistent decrease in diastolic blood pressure occurred following the intranasal administration of AVP, no such effect was observed in the analysis run on a subsample of eight subjects in which the P3 was also increased following the intranasal route of AVP administration.

Most important, AVP after intravenous administration remained ineffective despite the fact that plasma vasopressin concentrations after IV administered AVP (1.5 IU) in the main experiments were significantly higher than those

after the intranasal administration of AVP (20 IU). Lower doses of AVP administered IV, which induced plasma vasopressin concentrations comparable to that obtained after IN administration of AVP, also did not affect the ERP response. These data exclude that effects after IN administration depend on the AVP plasma concentration reached after this route of administration. On the contrary, the findings indicating a facilitation of CNS effects of AVP after IN administration provide functional evidence for the presence of a pathway for AVP or its signals from nasal mucosa to the brain, independent of blood-borne AVP.

Different anatomical routes have been proposed to account for the localization of various tracer substances to the brain after intranasal administration. Conclusive evidence for a direct extracellular pathway from the nose to the brain has been provided (Balin et al 1986; Itaya 1987). In those experiments, horseradish peroxidase administered intranasally in nonprimates and primates reached the fiber layer of the olfactory bulb within minutes after passing through patent intercellular clefts in the olfactory epithelium. Peroxidase diffused out of the olfactory fiber layer over time, presumably a consequence of bulk flow of cerebrospinal fluid.

Also, an intracellular transport of AVP could be possible. Tracer molecules have been shown to be endocytosed after intranasal administration and transported over time within olfactory sensory neurons to the glomular layer of the olfactory bulbs, where transsynaptic transfer of the molecules occurs (Broadwell and Balin 1985; Balin et al 1986; Baker and Spencer 1986); however, this route appears to take more time. Moreover, in the human olfactory bulb no specific binding sites for vasopressin have been found (Loup et al 1991). Considering the low speed of intracellular transport and the lack of specific binding sites of AVP in close proximity to the olfactory sensory neurons, we suppose the extracellular transport to be more relevant for the CNS effects of AVP after IN administration.

Regarding the xenobiotic metabolism in the nasal cavity, it cannot be ruled out that prior to its uptake the AVP molecule is degraded to centrally active fragments (Burbach et al 1983). The nasal epithelium contains a number of enzymes, such as P450 monooxygenases, esterases, and hydrolases, capable of degrading or altering a variety of compounds (e.g., Dahl and Hadley 1991). Also, presently it cannot be excluded that AVP effects are mediated via binding sites at the endings of the trigeminal and olfactory nerves, although it is not known whether these nerve terminals display specific receptors for AVP.

The present data do not exclude CNS effects of blood-borne AVP. A saturable active transport across the blood-brain barrier has been demonstrated for vasopressin (Zlokovic et al 1990), and persistent IV infusion of AVP for 9 hr has been shown to reliably affect CNS functioning in human subjects by reducing rapid eye movement sleep (Born et al 1991). In this context, the failure of AVP to affect ERP responses after IV administration in the present study, indicates a relative insensitivity of these routes to short-time increments of plasma vasopressin concentrations.

In summary, the present experiments demonstrate selective effects of AVP on electrophysiologic signs of CNS stimulus processing after intranasal administration. Intravenous infusion of different doses of AVP, inducing plasma vasopressin concentrations that were higher or comparable to those after intranasal administration, remained ineffective. The findings provide functional evidence for a facilitated access of vasopressin to the CNS after IN versus IV administration. Thus, the passage from nose to brain appears to offer a chance to directly influence CNS functioning in humans by peptide molecules, also for therapeutic purposes.

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