($P < 0.05$) than in control slices after 1–3 days of treatment, indicating that AP5 blocked AMPAfication. However, A/N began to increase after 4 days of treatment with AP5. Slices treated with AP5 for 8 or more days had significantly higher A/N ratios than did slices treated for 4 days or less ($P < 0.05$). After 8 days of AP5 treatment, slices became indistinguishable from untreated slices. The appearance of AMPAR responses after chronic NMDAR blockade is consistent with previous pharmacological[11–14] and genetic[9,10] experiments. Together, these findings indicate that additional NMDAR-independent mechanisms, recruited by chronic blockade of NMDAR activity, may be capable of driving AMPAfication.

We conclude that blockade of NMDAR activity can impair AMPAfication of glutamatergic synapses during development. However, our finding that AMPAfication of synaptic responses resumed after 4 days of chronic blockade of NMDAR function indicates that other mechanisms are involved. Although we cannot eliminate the possibility of an incomplete blockade by 200 μM AP5, this is unlikely given that similar results were obtained in animals completely lacking functional NMDARs[9]. It will be interesting to determine the nature of these mechanisms and whether other NMDAR-dependent processes, such as long-term potentiation and long-term depression, become NMDAR-independent after chronic NMDAR blockade. Lastly, this study shows that multiple 'reserve mechanisms' may exist for certain cellular processes, and that they may be recruited only under special conditions. This could explain why chronically disabling a gene or suppressing a protein's function often shows no obvious effects or an unexpected phenotype.

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Sniffing neuropeptides: a transnasal approach to the human brain

Jan Born[1], Tanja Lange[2], Werner Kern[2], Gerard P. McGregor[3], Ulrich Bickel[4] and Horst L. Fehm[2]

[1]*Departments of Neuroendocrinology and* [2]*Internal Medicine, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany*

[3]*Department of Physiology, University of Marburg, Deutschhausstrasse 2, 35037 Marburg, Germany*

[4]*Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, School of Pharmacy, 1300 Coulter Drive, Amarillo, Texas 79106, USA*

Correspondence should be addressed to J.B. (born@kfg.mu-luebeck.de)

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Neuropeptides act as neuronal messengers in the brain, influencing many neurobehavioral functions[1]. Their experimental and therapeutic use in humans has been hampered because, when administered systemically, these compounds do not readily pass the blood–brain barrier, and they evoke potent hormone-like side effects when circulating in the blood[2,3]. We administered three peptides, melanocortin(4–10) (MSH/ACTH(4–10)), vasopressin and insulin, intranasally and found that they achieved direct access to the cerebrospinal fluid (CSF) within 30 minutes, bypassing the bloodstream.

We selected the three peptides for their well-documented effects on brain functions including learning, memory, and body-weight regulation[1,4,5]. We administered the peptides intranasally to healthy humans (9 female, 27 male, 25–41 years of age), and the concentration of each peptide was measured within 80 minutes after administration in samples of CSF and systemic blood obtained through intraspinal (between L4 and L5) and intravenous (forearm) catheters. Catheterization was done two hours before the sampling period began.

Intranasal administration of each peptide resulted in an elevation of its concentration in the CSF (**Fig. 1**). We saw statistically significant peptide accumulation in the CSF within 80 minutes after administration with the higher dose of MSH/ACTH(4–10) (10 mg), with the higher and lower doses of vasopressin (80 and 40 IU) and with insulin (40 IU), as compared to pre-administration baseline concentrations and to concentrations in subjects administered sterile water as a placebo (**Table 1**). Also, a marginally significant ($P = 0.05$) increase in CSF concentration occurred between 60 and 80 minutes after administration of the lower dose of MSH/ACTH(4–10) (5 mg). Increases in the CSF concentration of each peptide varied considerably among subjects. For all three peptides, however, mean CSF concentrations began to rise within 10 minutes of intranasal administration. For MSH/ACTH(4–10) and insulin, peak levels were attained within 30 minutes after administration; for vasopressin, CSF concentrations continued to increase for up to 80 minutes after administration. For each peptide, concentrations did not return to baseline before the end of the 80-minute sampling period. More prolonged sampling in a subgroup of subjects receiving the higher doses of MSH/ACTH(4–10) and vasopressin showed that concentrations of peptides in the CSF levels were still above those in placebo-treated subjects 100–120 minutes after administration ($P < 0.03$ for MSH/ACTH(4–10), $P < 0.009$ for vasopressin).

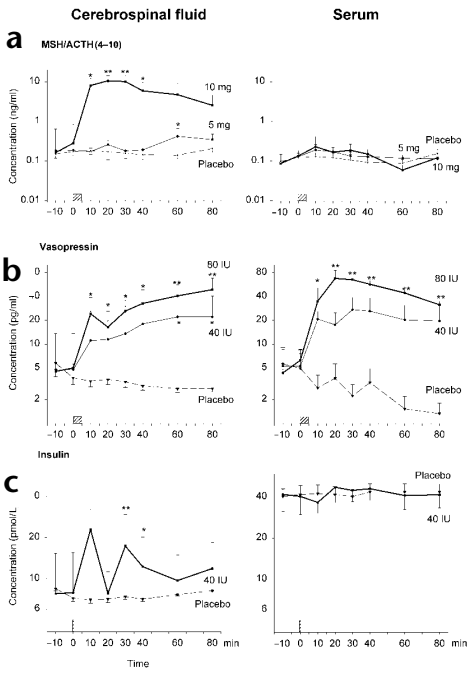

Fig. 1. Peptide accumulation in cerebrospinal fluid (CSF) and blood. Concentrations of (**a**) MSH/ACTH(4–10) (**b**) vasopressin and (**c**) insulin in CSF (left) and blood serum (right) from 10 min before to 80 min after their intranasal administration in humans. Doses were MSH/ACTH(4–10), 10 mg (thick solid line, $n = 5$) and 5 mg (thin solid, $n = 4$); arginine–vasopressin, 80 IU (thick solid, $n = 5$) and 40 IU (thin solid, $n = 4$); human insulin, 40 IU (thick solid, $n = 8$). Placebo (sterile water), thin dashed line ($n = 7$ for control of MSH/ACTH(4–10), $n = 5$ for control of vasopressin and insulin). Substances were administered with a nasal spray atomizer, with each puff containing defined amounts of MSH/ACTH(4–10) (0.25 or 0.5 mg), vasopressin (10 IU) or insulin (10 IU). Total doses were achieved by repeated puffs in each nostril every 30–45 s. Bars, period of peptide administration (for higher dose). Peptide concentrations were determined by radioimmunoassay (RIA) (MSH/ACTH(4–10)[11]; vasopressin, Mitsubishi Petrochemicals, Tokyo, Japan; insulin, Pharmacia, Uppsala, Sweden). No extraction procedure was used for CSF samples. Assay sensitivities were MSH/ACTH(4–10), 0.05 ng/ml; vasopressin, 0.2 pg/ml; insulin, 1.8 pmol/l. Cross-reactivity of the RIAs with naturally occurring related molecules was negligible (<0.1% with MSH/ACTH(4–9) or ACTH(1–24) for MSH/ACTH(4–10) RIA[11]; <0.04% with oxytocin, lysine–vasopressin or C-terminal metabolites for vasopressin RIA; <0.2% with C-peptide and insulin-like growth factor 1 and 2 for insulin RIA). RIAs were combined with reversed-phase high-performance liquid chromatography, confirming for each peptide that >90% of the immunoreactivity recovered in CSF represented the intact peptide. Means, s.e.m. and significance compared to placebo concentration (**, $P \leq 0.01$, *, $P < 0.05$, Mann-Whitney test, for baseline-adjusted values) are shown. Experiments were approved by the Ethics Committee of the University of Lübeck and informed consent was obtained from each participant.

Concurrent measurement of the concentrations in blood did not reveal a significant increase in MSH/ACTH(4–10) or insulin following intranasal administration of these peptides. In addition, there was no change in plasma glucose concentration after insulin administration ($P > 0.12$, for all comparisons). In contrast, the accumulation of vasopressin in CSF was accompanied by a distinct increase in plasma vasopressin levels, in agreement with previous observations[6]. The average increase in CSF concentrations of vasopressin correlated slightly but non-significantly with that in blood ($r = 0.55$, one-sided $P < 0.10$, across subjects and both doses).

Our data validate in humans the idea that intranasal administration allows peptides to penetrate into the CSF. These data corroborate previous human studies in which recordings of evoked brain potentials provided functional evidence for a facilitated access of neuropeptides to the brain after nasal delivery[7,8]. Animal studies also have shown that peptides (insulin, nerve growth factor) and larger molecules (e.g., horseradish peroxidase, viruses) accumulate in brain tissue after intranasal administration[3,9,10]. As the increased concentrations of MSH/ACTH(4–10) and insulin in the CSF after intranasal administration were not paralleled by any increase in the concentrations in the blood, and intranasal administration of insulin did not change blood glucose concentration, it is likely that the peptides entered the CSF directly, bypassing the bloodstream. In each case, an undetectable amount of peptide may have also reached the circulation. However, MSH/ACTH(4–10) is rapidly degraded in blood (half life <4 min[11]), and any insulin reaching the bloodstream would be masked by the endogenous hormone. Notably, in mice, the concurrent systemic injection of insulin has been found not to reduce a strong brain uptake of intranasally administered ^{125}I-labeled insulin[9]. Although these data support the hypothesis that intranasal peptide administration can result in uptake into the CSF independent of entry into the blood, other studies[12,13] in conjunction with our finding that intranasally administered vasopressin accumulated in plasma indicate that blood–brain transport may, to a certain extent, add to CSF uptake following nasal delivery.

Table 1. Accumulation of MSH/ACTH(4–10), vasopressin and insulin in CSF and blood serum.

	CSF			Serum		
	Mean	s.e.m.	*P*	Mean	s.e.m.	*P*
MSH/ACTH(4–10)	**AUC (ng/ml) × min**			**AUC (ng/ml) × min**		
Placebo	7.45	8.98		8.80	1.41	
MSH/ACTH(4–10), 5 mg	21.53	7.11	0.30	10.91	0.26	0.48
MSH/ACTH(4–10), 10 mg	514.49	195.4	0.004	10.98	2.98	0.62
Vasopressin	**AUC (pg/ml) × min**			**AUC (pg/ml) × min**		
Placebo	254.4	65.6		207.4	202.0	
Vasopressin, 40 IU	1,319.1	821.8	0.05	1,674.0	931.8	0.14
Vasopressin, 80 IU	2,481.9	732.4	0.009	3,749.8	348.6	0.009
Insulin	**AUC (pmol/l) × min**			**AUC (pmol/l) × min**		
Placebo	603.2	34.6		3,410.5	106.1	
Insulin, 40 IU	1,091.1	219.8	0.028	3,414.3	276.8	0.22

Concentrations of the peptides expressed as area under curve (AUC; using the trapezoid method) within 80 min after intranasal administration. $P < 0.05$ indicates significance in comparison with concentrations after placebo administration. Significance of accumulations was also confirmed in comparisons of average post-administration increases with pre-administration baseline concentrations.

Two routes have been proposed for the direct passage of peptides from the nose to the brain: an intraneuronal and an extraneuronal pathway[3,10,14]. Intraneuronal transport involves the internalization of the peptide into olfactory neurons, followed by axonal transport. However, this route presents a greater risk of proteolysis (resulting from lysosomal degradation) than does extraneuronal transport, and requires hours for substances to reach the olfactory bulb[3,14]. It therefore seems more plausible that peptide molecules travel by the extracellular route, passing through patent intercellular clefts in the olfactory epithelium to diffuse into the subarachnoid space[3,14]. AUC calculations (**Table 1**) are suggestive of an inverse relationship between accumulation in the CSF and peptide molecular weight (MWs: MSH/ACTH(4–10), 962.1; vaso-

pressin, 1,084.2; insulin, 5,808.0), although other factors, such as lipophilicity and degree of ionization, probably also affect peptide access to the brain[3]. A rapid accumulation of peptides in cerebral and spinal CSF and in brain tissue (within 10–20 minutes of intranasal administration) has been seen in animals and is also suggested by observations in patients[3,7,9]. Although the extent of peptide uptake from CSF into human brain tissue is not known, animal studies have shown significant uptake even in more interior brain regions, such as the amygdala[10].

Our data cannot be taken to establish that intranasal administration results in greater CSF uptake of peptides than does intravenous administration. We administered fairly large doses of peptide, because with intranasal administration, substantial amounts of a compound may simply pass through the nose without being absorbed. In the case of insulin, for example, previous studies suggest that brain uptake from plasma is at least comparable to that resulting from nasal delivery[12,13]. This conclusion does not, however, detract from our finding that intranasal administration can deliver neuropeptides to the brain without uptake into the circulation. The potential usefulness of nasal administration derives from the fact that biologically effective concentrations of neuropeptides can be achieved in the human brain without strong systemic, hormone-like side effects. Such effects limit the systemic administration of peptide to amounts too small to have substantial effects in the brain. Nasal delivery may be useful in the treatment of brain diseases, particularly those involving dysfunction of neuropeptide signaling, such as Alzheimer's disease and obesity[5,9,10]. Although this theory has received support from human studies showing beneficial effects on sleep and body fat mass after prolonged intranasal administration of vasopressin and MSH/ACTH(4–10), respectively, it remains to be proven in clinical trials[7,15]. Nevertheless, the utility of the intranasal route of peptide administration remains to be proven in clinical trials.

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