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should be addressed to
R.H. (renehurlemann@
me.com)

Elevated cerebrospinal fluid and blood concentrations of oxytocin following its intranasal administration in humans

Nadine Striepens[1,2], Keith M. Kendrick[4], Vanessa Hanking[1,2], Rainer Landgraf[5], Ullrich Wüllner[3], Wolfgang Maier[1,6] & René Hurlemann[1,2]

[1]Department of Psychiatry, University of Bonn, 53105 Bonn, Germany, [2]Division of Medical Psychology, University of Bonn, 53105 Bonn, Germany, [3]Department of Neurology, University of Bonn, 53105 Bonn, Germany, [4]Key Laboratory for Neuroinformation, School of Life Science and Technology, University of Electronic Science and Technology of China (UESTC), 610054 Chengdu, P. R. China, [5]Max Planck Institute of Psychiatry, 80804 Munich, Germany, [6]German Center for Neurodegenerative Diseases (DZNE), 53175 Bonn, Germany.

There has been an unprecedented interest in the modulatory effects of intranasal oxytocin on human social cognition and behaviour, however as yet no study has actually demonstrated that this modality of administration increases concentrations of the peptide in the brain as well as blood in humans. Here using combined blood and cerebrospinal fluid (CSF) sampling in subjects receiving either 24 IU of oxytocin (n = 11) or placebo (n = 4) we have shown that oxytocin levels significantly increased in both plasma and CSF. However, whereas oxytocin plasma concentrations peaked at 15 min after intranasal administration and decreased after 75 min, CSF concentrations took up to 75 min to reach a significant level. Moreover, there was no correlation (r = <0.10) between oxytocin plasma and CSF concentrations. Together, these data provide crucial insights into the plasma and CSF kinetics of intranasally administered oxytocin.

The neuropeptide oxytocin (OXT) has recently emerged as playing a key role in human social cognition and behaviour. Current perspectives on the neural circuits underlying OXT effects on human social cognition and behaviour suggest a modulation of regions involved in social-emotional processing, with altered amygdala activation in response to facial expressions of emotion being one of the most consistent findings[1–3]. Increasingly, clinical studies have generated unprecedented interest in the therapeutic targets and potential benefits of intranasal OXT application for neuropsychiatric disorders characterised by social dysfunction, such as social anxiety, autism, and schizophrenia[4].

Despite extensive research and very promising results, however, little is known about the pharmacokinetic characteristics of intranasal OXT application. Investigations into the potential effects of OXT in humans were initially hampered by the knowledge derived from animal-based work that approximately 0.01% of the peptide crossed the blood-brain barrier (BBB) following intravenous injection[5] and that peripherally administered peptides have a very brief half-life[6]. Consequently, the most convincing animal studies of functional effects of OXT have therefore used intracerebroventricular or intracerebral administration routes. However, recently, intranasal application of OXT has been shown to increase the OXT content in microdialysates sampled within the dorsal hippocampus and the amygdala in rats and mice, which was preceded by a rather rapid rise in plasma concentration[7]. Moreover, it has been shown that insulin, melanocyte-stimulating hormone, and vasopressin can all cross the BBB and increase cerebrospinal fluid (CSF) as well as blood concentrations within 15–30 min after intranasal administration[8]. Furthermore, intranasal delivery has been used to transport a wide range of therapeutics into the CNS, for example neurotrophines (e.g., insulin-like growth factor [IGF]-1), neuropeptides (e.g., hypocretin-1 and exendin), cytokines (e.g., erythropoietin), and carbamazepine[9,10]. The direct-to-the-brain delivery of therapeutics via the nasal route may engage extraneuronal/perineuronal routes along trigeminal or olfactory nerve pathways; other mechanisms of entry such as bulk flow, transport via lymphatic channels, intraneuronal transport, and active or passive transport from vasculature are also possible[4]. Moreover, intranasally delivered OXT may cause some of its central effects by stimulating the endogenous OXT system, which secretes OXT into the peripheral circulation but also distributes it within the brain – via targeted synaptic/dendritic release as well as less targeted long-distance diffusion from hypothalamic release sites[2].

Preliminary results have suggested that OXT nasal spray may increase peptide levels in both peripheral blood and saliva. Elevated OXT plasma concentrations peaking at 10–40 min after intranasal administration of OXT (30 IU) have been documented in three male volunteers[11]. Two other studies have shown increases in OXT plasma concentrations 30–40 min after intranasal administration of 24 IU[12,13]. OXT saliva concentrations have also been reported to increase dramatically and for up to 7 h after intranasal administration of either 16 or 24 IU[14], but may not be a valid biomarker when measured by immunoassay[15].

In the present study, we have examined whether OXT concentrations increase in the CSF as well as blood following intranasal administration. We have also investigated the correlation between altered plasma and CSF concentrations.

Results

As expected, there was an increase in plasma concentrations following OXT intranasal treatment (one-way ANOVA $F = 14.408$, $p < 0.001$; df = 6; Figure 1A). Post-hoc Bonferroni tests showed that OXT plasma levels were elevated at 15, 30, 45, and 60 min (all $p < 0.001$) compared to baseline. Concentrations at the 75 and 90 min

time points were lower than those at 15, 30 and 45 min (all $p < 0.05$) and those at 90 min were lower than at 60 min ($p < 0.05$). A one-way ANOVA showed no changes across time points in the PLC treated group ($F = 0.782$; $p = 0.595$; df = 6). Plasma concentrations did not differ between the two groups at baseline (OXT, mean $\pm$ SD, 8.0 ± 4.3 pg/ml; PLC, mean $\pm$ SD, 5.9 ± 2.8 pg/ml, $p > 0.05$ t-test) or at 90 minutes (OXT, 12.3 ± 6.0 pg/ml; PLC, 5.0 ± 1.6 pg/ml; $p > 0.05$) after drug application.

There was no correlation between CSF and plasma concentrations of OXT across the coincident sampling time points (Spearman correlation $r = 0.10$, $p = 0.714$). In general, mean CSF levels of OXT in the PLC group were much higher than those in plasma (mean $\pm$ SD CSF, 19.1 ± 2.4 pg/ml; plasma, 6.2 ± 2.4 pg/ml; $t = -6.097$, $p = 0.009$).

Mean CSF concentrations of OXT significantly differed between the sampling time points and treatment groups ($F = 6.434$, $p = 0.009$). Specifically, post-hoc Bonferroni tests showed that CSF concentrations were elevated following intranasal OXT administration after 75 min compared with the PLC group ($p = 0.024$) or with the group of OXT-treated subjects sampled at 45 min ($p = 0.011$) (Figure 1B). Notably, CSF levels of OXT in subjects sampled at 45 and 60 minutes post nasal spray application did not differ from the PLC group ($p > 0.05$). Moreover, there was no difference between CSF levels of OXT in subjects sampled at 60 and 75 minutes post OXT treatment ($p > 0.05$).

Discussion

The present study is the first to show that intranasal administration of OXT at the 24 IU dose which is given in the majority of behavioural and neuroimaging studies leads to increased OXT concentrations in the human CSF. However, at this dose, increases are relatively modest and not significant until 75 min after intranasal administration. On the other hand OXT increases in plasma are much greater and more rapid but have returned to baseline by 75 min after intranasal treatment. Thus, overall there is no significant correlation between CSF and plasma concentrations of the peptide. We also found that basal concentrations of OXT are higher in CSF than those in plasma.

Our finding of elevated OXT concentrations in the CSF after intranasal OXT application is in line with the recent demonstration of elevated OXT content in the extracellular fluid within the septum and dorsal hippocampus of rats and mice monitored by intracerebral microdialysis, which peaked between 30 and 60 min after intranasal application of OXT and was accompanied by a sharp but independent rise in plasma OXT[7]. It is also in line with numerous reports of modulatory effects of OXT on human social cognition and behaviour, particularly in the domain of social-emotional processing[1–3]. While our results are in general agreement with the only previous report on effects of intranasal administration of other neuropeptides on CSF and plasma concentrations in humans[8], the time-courses and magnitude of changes in the two studies are different. Specifically, Born and colleagues[8] reported up to 4-fold significant increases in CSF concentrations following administration of arginine vasopressin (AVP) after only 10 min at a dose of 80 IU, although with a dose of 40 IU the increase took up to 60 min to occur. Comparing the two sets of results one possible interpretation is that high doses of intranasal peptide administration result in both a more rapid and greater concentration change in the CSF. Perhaps this is a little surprising given that circulation of CSF in the brain should be relatively constant; however, when an intranasally given peptide penetrates into the CSF at higher concentrations, it may create a greater concentration gradient which could likely accelerate the occurrence of increases in the remotest part of the system, the lumbar spine. Since we found a more than 60% increase in peptide concentrations after 75 min it seems possible that at later sampling time points concentrations might have increased even further. Interestingly, a recent study involving mon-
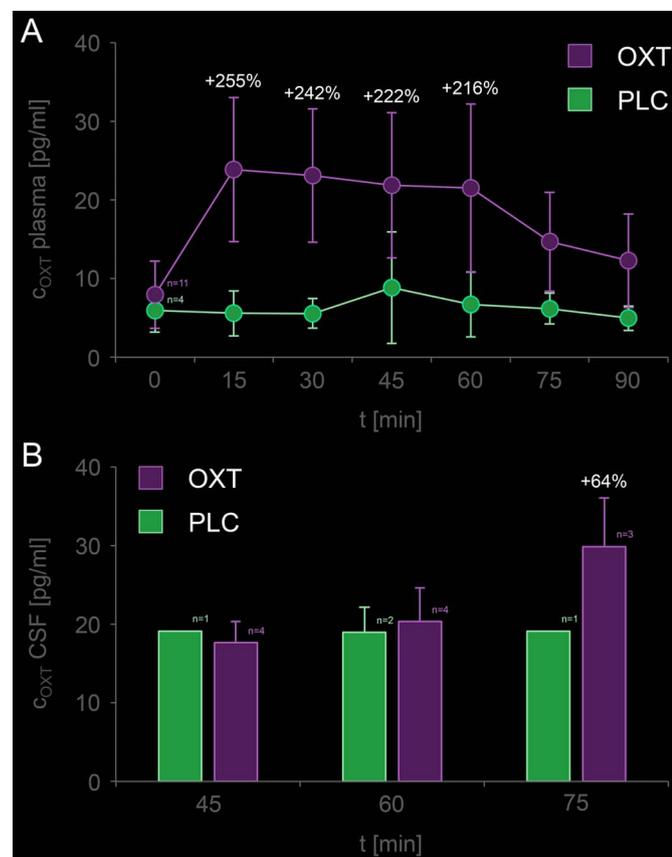
Figure 1 | Plasma and CSF kinetics of oxytocin (OXT) administered intranasally at a dose of 24 IU. (A) Shown are OXT plasma concentrations (mean $\pm$ SD) immediately before (baseline, 0 min) and after intranasal OXT or placebo (PLC) treatment. OXT plasma concentrations reached a plateau after 15 min and decreased after 60 min. Given for each time point in this period is the percentage increase (corrected for the lowest OXT concentration at 0 min) over PLC mean (0–90 min). (B) Shown are oxytocin (OXT) cerebrospinal fluid (CSF) concentrations following intranasal oxytocin (OXT) or placebo (PLC) treatment. CSF levels took up to 75 min to reach a significantly increased level in the OXT group. Given is the percentage increase (corrected for the lowest OXT concentration at 45 min) over PLC mean (45–75 min). Abbreviation: c_{OXT}, oxytocin concentration.

keys reported that following intranasal/mouth administration of OXT at a dose of 25 IU CSF concentrations increased from around 20 to 50 pg/ml after 35 min; however, CSF sampling was done higher up in the CSF system by cervical puncture, which might contribute to the observed discrepancy between studies[16].

The mechanism by which intranasal administration of peptides leads to increased CSF and blood levels of the peptide remains unclear. Intraneuronal and/or extraneuronal pathways could be involved. For instance, the peptide could be taken up by olfactory neurons and conveyed within these neurons via axonal transport[9]. However, this would take several hours. Thus, extraneuronal pathways involving trigeminal[10] and/or subarachnoid[17] routes appear more likely. Other mechanisms of entry such as bulk flow, transport via lymphatic channels, and active or passive transport from vasculature are also possible[4]. On the other hand, increased central levels of OXT after intranasal administration could also occur indirectly through afferent feedback signals from the periphery, which may stimulate endogenous release within the brain but also secretion into peripheral circulation, thereby further elevating blood OXT levels. Thus the present findings do not necessarily represent evidence of a direct nose-to-brain penetration of OXT. Increased concentrations in blood may result from absorption by the heavily vascularized nasal mucosa, which drains through both fenestrated epithelium and via several facial veins into the peripheral circulation[4]. Indeed, a study in rodents found that constriction of these vessels by phenylephrine significantly reduced blood concentrations of neuropeptides given intranasally without reducing CSF concentrations[18].

The lack of correspondence between CSF and plasma OXT concentrations in our study supports previous observations in monkeys[19], sheep[5], and humans[20] and suggests some degree of caution in assuming that blood OXT concentrations are always an accurate reflection of those occurring in the brain[20]. The higher basal concentrations of OXT we found in CSF compared to plasma is likely to be due to its diffusion from the extracellular fluid, where it is highly concentrated[7], and its 30–40-fold increased half-life in CSF[19]. Other animal-based studies have also reported higher and more prolonged increased concentrations of OXT in CSF compared to blood[5]. From these observations it is clear that OXT concentrations are constantly high in the CSF and that a paracrine mode of action is quite likely. This may help to explain why a simple intranasal application that elevates brain CSF concentrations can promote complex behavioural changes by targeting receptors simultaneously across widely distributed brain regions. A limitation in our current study however was that the lack of correspondence between CSF and plasma OXT concentration was only explored 45, 60 and 75 min after intranasal administration. It is conceivable that CSF OXT levels could have risen significantly and declined again before 45 min in some subjects in the current study, although the previous study showing rapid appearance of AVP concentration at a 80IU dose also showed that elevated concentrations were maintained for the remaining 70 min of the sampling period[8]. A similar long lasting effect on brain OXY concentrations was also shown in rats[7] and both studies generally support evidence for a prolonged half-life of OXT and AVP in CSF[19]. Nevertheless, future studies are needed to evaluate the correspondence of plasma and CSF concentrations of OXT following intranasal application outside the limited time window of the current study.

The majority of previous studies using single-dose intranasal OXT administration have used the same 24 IU dose that we chose for the current study[1–4]. These studies generally wait at least 45 min after administration before conducting behavioural and neuroimaging experiments which can last an hour or more. Given the time-course of the plasma and CSF changes we have observed it is clear that by 75 min after intranasal administration (i.e. in the middle of many tasks) OXT concentrations are only just increasing in CSF (at least at the lumbar spine level) and that by this time changes may have disappeared in the blood. Thus, there is some support for considering

delaying starting tasks following this dose of OXT a little longer than 45 min. Alternatively, given the possibility of reduced time-course effects of increased doses of intranasal OXT experiments could start earlier than 45 min if higher doses are used. However, this needs further experimental confirmation.

There are several limitations to the present study. The first and major limitation concerns the small sample size with eleven individuals in the OXT group and four in the PLC group. However, previous studies[8] included only four to seven volunteers, and it is difficult to obtain ethical approval for large-scale studies. We also observed very consistent OXT concentrations across subjects. Nevertheless, studies involving low numbers of subjects do require some caution until further replication will increase confidence. Secondly, the study only measured CSF concentrations at one time point in each subject (45, 60 or 75 minutes) and we could only use OXT CSF concentrations in the PLC group to provide a baseline. We cannot rule out that the subjects showing increased CSF levels at 75 min might by chance have been those with better uptake into the cerebroventricular system and might therefore also have shown elevated levels at the 45 or 60 min time points. Measuring the CSF concentration at one time point in each subject was imposed because our subjects were undergoing specific diagnostic sampling. However, OXT concentrations in PLC treated subjects and in the OXT treated subjects at the 45 and 60 min time points were very similar. Further, the final CSF sample was taken 75 minutes after intranasal OXT administration and was the only one to show significantly increased OXT concentrations. It is possible that at later time points OXT concentrations would have increased even further, perhaps to the 3–4 fold level observed in other studies[8,16]. Future studies are needed to evaluate the precise duration of elevated OXT concentrations in CSF after intranasal OXT treatment, although since the total volume of CSF is renewed every 4–5 h in humans[21] it is unlikely that effects could outlast this period of time. Finally, we do not know precisely how long it actually takes for intranasal oxytocin to reach the lumbar spine as opposed to the brain ventricles although it is unlikely that this delay is more than 5–10 min or so given a 1–5 mm/s transport speed[21,22].

In conclusion, to our knowledge this is the first study that provides evidence that intranasally administered OXT elevates OXT concentrations in human plasma and CSF and that kinetics of changes in these compartments are different.

Methods

Participants. Adult male volunteers (OXT group: n = 11, age range, 24–64 years, mean age ± SD, 46.7 ± 12.0 years; placebo (PLC) group: n = 4, age range 19–63 years, mean age, 42.5 ± 18.9 years) participated in this study after providing written informed consent in accordance with the Declaration of Helsinki. The study protocol had been approved by both the institutional ethics review board (IRB) of the Medical Faculty of the University of Bonn and the German Federal Institute of Drugs and Medical Devices (BfArM). At the time this study took place, all subjects were inpatients of the Department of Neurology of the University of Bonn and underwent routine lumbar puncture (LP) for diagnostic purposes due to polyneuropathy (n = 11), peripheral facial nerve paresis (n = 3), or to exclude a subarachnoid hemorrhage (n = 1). Two patients were subsequently excluded from our study, one due to diagnosis of multiple sclerosis (encephalomyelitis disseminata) and another one due to problems with CSF sampling. All participants included in our study were free of current or past endocrine or psychiatric disorders, as assessed by medical history and a Structured Clinical Interview for DSM-IV axis I (SCID-I)[23] and axis II disorders (SCID-II)[24]. Volunteers were naive to prescription-strength psychoactive medication and had not taken any over-the-counter psychoactive medication in the past 4 weeks. Subjects were asked to avoid the intake of alcohol for at least 24 h prior to this study.

Neuropsychological screening. All participants completed a comprehensive neuropsychological test battery (Table 1). Specifically, neuropsychological testing before study enrollment included the LPS 4 ('Leistungspruefsystem Subtest 4')[25] to assess nonverbal reasoning IQ, the MWT-B ('Mehrfach-Wortschatz-Intelligenztest Teil B')[26] to assess verbal IQ based on lexical decisions, and the trail-making test (TMT)[27] part A and B to assess visual attention and task-switching performance. Furthermore, depressive symptoms were assessed by the self-report BDI (Beck's Depression Inventory, Version II)[28].

Table 1 | Demographics and neuropsychological performance

	OXT group (n = 11)	PLC group (n = 4)	P value
Age, years	46.7 (12.0)	42.5 (18.9)	.612
LPS-4 [a]	25.5 (3.9)	22.3 (5.7)	.229
MWT-A [b]	28.9 (3.2)	27.8 (5.7)	.569
TMT-A [c]	25.5 (3.3)	31.3 (8.3)	.064
TMT-B [c]	87.7 (17.6)	86.5 (25.3)	.916
BDI [d]	1.9 (1.4)	1.8 (1.0)	.836

Nonverbal reasoning IQ was assessed by the [a]LPS (Leistungsprüfsystem) subtest 4 (maximum possible score 40)[25]; verbal IQ based on lexical decisions was assessed by the [b]MWT-A (Mehrfachwahl-Wortschatz-Intelligenz-Test Teil A) (maximum possible score 37)[26]; visual attention and task-switching were assessed using the [c]TMT-A and TMT-B (Trail-making test A, B) (results are displayed in seconds)[27]; depressive symptoms were assessed by the self-report [d]BDI (Beck's Depression Inventory, Version II)[28]. Two-sample t-tests confirmed no significant (p < 0.05) pre-treatment differences in demographic and neuropsychological characteristics between the placebo (PLC) and oxytocin (OXT) treated groups.

Study design. In this double-blind, randomized, placebo-controlled, between-subject study we measured OXT concentrations in plasma and CSF after intranasal OXT or PLC administration. Subjects were randomly assigned to receive either intranasal OXT (24 IU; Syntocinon spray manufactured by Novartis, Rotkreuz, Switzerland; 3 puffs per nostril, each with 4 IU OXT) or PLC (sodium chloride solution) treatment. To control dosing and absorption all subjects administered the nasal spray in a head upright position, closed one nostril with one finger while administering the spray to the other nostril and sniffing during administration. The bottle was inserted 1 cm into the nostril with an administration angle of 45 degrees into the nose. According to the guidelines for intranasal OXT administration by Guastella et al. subjects self-administered the nasal spray under supervision of the experimenter and after receiving verbal instructions and observing a demonstration by them[29]. CSF was taken by lumbar puncture (LP) in a sitting position at 45 min (OXT, n = 4; PLC n = 1), 60 min (OXT, n = 4; PLC, n = 2) or 75 min (OXT, n = 3; PLC, n = 1) after intranasal administration. To control for circadian variability, all LPs were carried out at the same time of day (01:00–03:45 p.m.). After taking CSF for routine diagnostic purposes (leukocytes and erythrocyte cell count, glucose, lactate, and total protein content), a further volume of 10–12 ml was collected in polypropylene tubes for OXT assay. Plasma levels of OXT were analysed based on blood samples drawn via an intravenous catheter at 7 consecutive time points over a time window of 95 min: the first one 5 min before drug application (baseline) and then again at 15, 30, 45, 60, 75 and 90 min after intranasal treatment. Blood was collected in 2.7 ml lithium-heparin cell preparation tubes. To inhibit activity of proteinases, aprotinin (Sigma-Aldrich, St. Louis, MO) was immediately added to blood and CSF samples (aprotinin 0.1 mg/ 1 ml) and centrifuged within one hour after sampling for 15 min at 1600 g and 4°C to remove cells. The supernatant was aliquoted into Eppendorf tubes and frozen at −80°C until assays were performed. CSF samples containing more than 500 erythrocytes per μl (prior to centrifugation) were excluded from the analysis.

Endocrine measures. CSF and plasma OXT was extracted and quantified by a highly sensitive and specific radioimmunoassay (RIAgnosis, Munich, Germany)[7,20].

Statistics. Statistical analyses were performed using IBM SPSS statistics 20 software (IBM, Armonk, NY). After testing for normal distribution (Shapiro-Wilks), one-way parametric ANOVA tests were used to analyse differences in CSF and blood concentrations of OXT at different time points following the intranasal treatments. When significance ($p < 0.05$) was reached, post-hoc Bonferroni tests were used to compare concentrations at individual time points. Correlations between plasma and CSF concentrations across all time points were analysed using a Spearman rank test. Differences in baseline OXT concentrations in blood and CSF in PLC-treated subjects were analysed using paired t-tests.

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Author contributions

N.S., R.H. and W.M. designed the study and wrote the protocol. N.S., V.H., and U.W. carried out the study. N.S., R.L. and K.M.K. analyzed data. All authors contributed to and have approved the final version of the manuscript.

Additional information

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