Salivary levels of oxytocin remain elevated for more than two hours after intranasal oxytocin administration

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Abstract

OBJECTIVE: This is the first study investigating whether levels of oxytocin in saliva remained elevated after intranasal oxytocin administration for the duration of an experiment (in which neurobehavioral effects of oxytocin were observed) taking more than two hours.
METHODS: Oxytocin levels were measured in saliva samples collected from 57 female participants right before (T0), approximately 1¼ h (T1), and approximately 2¼ h (T2) after intranasal administration of 16 IU of oxytocin or a placebo, using a double-blind, within-subjects design.
RESULTS: Average levels of oxytocin did not differ between conditions before use of the nasal spray, markedly increased only after oxytocin administration, and were still elevated after 2¼ h.
CONCLUSION: Salivary levels of oxytocin remained persistently elevated over the course of our experiment, i.e. for more than two hours after intranasal oxytocin administration and over a time-period in which neurobehavioral effects of oxytocin are commonly observed. This suggests that salivary concentrations may be a valuable biomarker for oxytocin, and may help to explain its effects on brain activity, information processing, and behavior.

ORIGINAL ARTICLE

INTRODUCTION

Oxytocin is a neuropeptide that is synthesized in magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus that project to the posterior pituitary from which oxytocin is released into the bloodstream. In addition, neurons in the PVN project to various limbic, mid- , and hindbrain structures (e.g., hippocampus, amygdala, and nucleus accumbens) expressing oxytocin receptors. Within the brain, oxytocin can act both as a neurotransmitter and as a neuromodulator (Landgraf & Neumann 2004; Skuse & Gallagher 2009). In humans as well as

other mammals oxytocin plays an important role in parturition and lactation, is involved in regulation of the hypothalamic-pituitary-adrenal axis, and facilitates reproductive and maternal behavior, infant attachment, and social information-processing and behavior (e.g., Campbell 2008; Carter 2003; Feldman *et al.* 2007; Insel 1992; Naber *et al.* 2010; Parker *et al.* 2005; for reviews see Heinrichs *et al.* (2009), MacDonald & MacDonald (2010), Bartz *et al.* (2011), and Galbally *et al.* (2011); for a meta-analysis of experimental studies with humans see Van IJzendoorn & Bakermans-Kranenburg (2012).

Levels of circulating oxytocin have been successfully measured in blood plasma (e.g., Grewen *et al.* 2010; Light *et al.* 2005; Morhenn *et al.* 2008; Tops *et al.* 2007), urine (e.g., Gray *et al.* 2007), saliva (e.g., Grewen *et al.* 2010; Holt-Lunstad *et al.* 2008; White-Traut *et al.* 2009), and cerebrospinal fluid (e.g., Heim *et al.* 2008). It is well known that elevations of oxytocin levels in blood (in which it has a half-life of only a few minutes) after exogenous administration of the neuropeptide do not adequately reflect the time-range of its neurobehavioral effects (that may last for several hours), but little is known about oxytocin in other bodily fluids (McEwen 2004). Because samples of saliva can be collected easily and non-invasively, measuring salivary levels of oxytocin may be particularly promising. We therefore measured oxytocin levels in saliva samples collected over the course of an experiment (focusing on neural responses to emotionally relevant stimuli) with female participants to investigate for the first time whether oxytocin levels would be elevated in saliva for the entire duration of the experiment, i.e. for more than two hours after intranasal oxytocin administration.

MATERIAL AND METHODS

Participants

A total of 59 female undergraduate students, aged 18–30 years (M=20.54, SD=2.89), took part in the experiment. Two participants were excluded, because they completed only one condition (placebo or oxytocin). The final sample thus consisted of 57 participants (aged 18–30 years, M=20.51, SD=2.90). They were paid 50 Euros for participation. Exclusion criteria included colorblindness, smoking, alcohol and drug abuse, neurological and psychiatric disorders, pregnancy, breastfeeding, and use of medication except oral contraceptives (use of oral contraceptives was recorded as a covariate). The study was approved by the ethics committee of the Leiden University Medical Center.

Procedure

Participants were asked to come to our laboratory for two experimental sessions, separated by approximately four weeks. To minimize influences of diurnal variations in oxytocin levels, all sessions took place in the afternoon (starting between 12:00 and 3:00 p.m.). Participants were instructed to abstain from alcohol and excessive physical activity during the 24 hours before the start of each session, and from caffeine on the day the session took place.

Informed consent was obtained at the beginning of the first session. Participants were not informed about the potential effects of oxytocin under investigation, only about the possible side effects they might experience (as was required by the ethics committee).

At the start of each session (T0), a saliva sample was collected and participants completed a number of questionnaires. The participants then received nasal spray containing either 16 IU of oxytocin or a placebo (saline solution). All participants received both substances once, either the placebo during the first session and oxytocin during the second, or oxytocin during the first session and the placebo during the second. The order of administration was counterbalanced across participants and unknown to both the participant and the experimenter. Participants were then fitted with an electrode net after which they completed a modified Eriksen flanker task (Eriksen & Eriksen 1974). While performing this task, participants were presented with feedback after every response. Pictures of emotional faces (happy or disgusted) were presented in green after correct responses and in red after errors. Neurobehavioral analyses focused on effects of both oxytocin and other variables on event-related potential (ERP) responses to these facial feedback stimuli (see Huffmeijer *et al.* 2011). The flanker task began approximately 45 minutes after oxytocin or placebo administration. Halfway through the task (T1, approximately 1¼ hours after nasal spray administration) and after completion of the task (T2, approximately 2¼ hours after nasal spray administration) saliva samples were collected and participants completed several questionnaires. Here, we present data regarding salivary oxytocin (for a description of neurobehavioral effects of oxytocin during the present experiment see Huffmeijer *et al.* 2011).

Salivary oxytocin

For each sample at least 1 mL of unstimulated saliva was collected into 1.8 mL cryotubes using the passive drool method. Samples were immediately frozen and were stored at –20 °C until batch assay. Level of oxytocin (OT) in saliva was assayed using a commercially available kit as per the method previously described (Grewen *et al.* 2010; Holt-Lunstad *et al.* 2008). Prior to the enzyme immunoassay procedure, in keeping with the manufacturer's strong recommendation, an extraction step was performed based on instructions accompanying the EIA kit available in February 2011 (ADI-900-153, Enzo Life Science, Plymouth Meeting, PA). The result of this extraction was to concentrate the sample 3.2 times, increase precision and reduce matrix interference. OT extraction efficiency was 93%, which was determined by spiking with a known amount of hormone and extracting this known amount

along with the other samples. OT levels in extracted saliva were then quantified using the OT EIA, in which the endogenous OT hormone competes with added OT linked to alkaline phosphatase for OT antibody binding sites. After overnight incubation at 4 °C, the excess reagents were washed away and the bound OT phosphatase was incubated with substrate. After 1 hour this enzyme reaction, which generates a yellow color, was stopped and the optical density (OD) was read on a Sunrise plate reader (Tecan, Research Triangle Park, NC). The intensity of the color at 405 nm is inversely proportional to the concentration of OT. The hormone content (in pg/mL) was determined by plotting the intensity of OD of each sample against a standard curve. Following correction for extraction, the lower limit of sensitivity was 1.25 pg/mL. Less than 1% of the samples fell below the lower level of sensitivity (4 out of 348). These values were subsequently replaced with the lowest detectable level of 1.25 pg/mL. The intra- and inter-assay coefficients of variation were 7.35% and 8.51% respectively. The manufacturer reports that cross-reactivity with similar mammalian neuropeptides is less than 1%.

The mean raw values of salivary oxytocin were 7.75 (*SD*=4.96; placebo condition) and 7.31 (*SD*=4.34; oxytocin condition) at T0, 8.41 (*SD*=18.34; placebo condition) and 186.19 (*SD*=159.48; oxytocin condition) at T1, and 5.30 (*SD*=3.33; placebo condition) and 148.47 (*SD*=144.33; oxytocin condition) at T2. For three participants one oxytocin value (T0 for one, T1 for a second, T2 for the third, all placebo condition) was considered an outlier (z>3.29) within the respective time point and condition. For statistical analysis these values were replaced with the highest value occurring at that respective time point and condition among the remaining participants. In addition, values of two participants at T1 and another participant at T2 of the oxytocin condition fell too far outside the normal curve to be computed reliably. These missing values were replaced with the mean value of the respective time point and condition across the remaining participants. Because the distributions of oxytocin values were skewed, we computed the natural logarithm of the raw values.

Analyses

Statistical analyses were performed using SPSS 17 software. To test whether oxytocin levels in saliva increased after oxytocin administration, a repeated measures GLM analysis was performed with condition (placebo vs. oxytocin) and time (T0, T1, T2) as within subjects factors. To control for potential influences of order of administration (placebo first vs. oxytocin first) and use of oral contraceptives (used vs. not used) on circulating levels of oxytocin, these two variables were included as additional (between subjects) factors in a second GLM analysis. Greenhouse-Geisser corrections were performed when necessary.

RESULTS

The ln-transformed average levels of oxytocin at the different time-points during the placebo and oxytocin conditions are plotted in Figure 1. The GLM analysis revealed significant main effects of condition, $F(1,56)=299.10$, $p<0.01$, $\eta^2=0.84$, and time, $F(1.77, 98.87)=98.18$, $p<0.01$, $\eta^2=0.64$, qualified by a significant interaction between condition and time, $F(2,112)=122.12$, $p<0.01$, $\eta^2=0.69$. As can be seen in Figure 1, average levels of oxytocin were virtually the same in both conditions before nasal spray administration (T0, *M*=1.86, *SD*=0.60 [placebo condition], *M*=1.83, *SD*=0.57 [oxytocin condition]) and markedly increased after oxytocin administration. Including order of administration and use of oral contraceptives as additional factors in the analyses did not affect the results: both main effects (condition: $F(1,54)=242.13$, $p<0.01$; time: $F(1.77, 95.45)=81.43$, $p<0.01$) and the interaction between condition and time ($F(2,108)=108.58$, $p<0.01$) remained significant, and no significant effects involving order of administration (all Fs ≤1.04, ps>0.10) or use of oral contraceptives (all Fs ≤2.67, ps>0.10) were obtained.

Repeating these analyses with the ln-transformations of all original oxytocin values (i.e., without replacing the three outliers) as dependent variable, and excluding the three participants with missing values, did not change results.

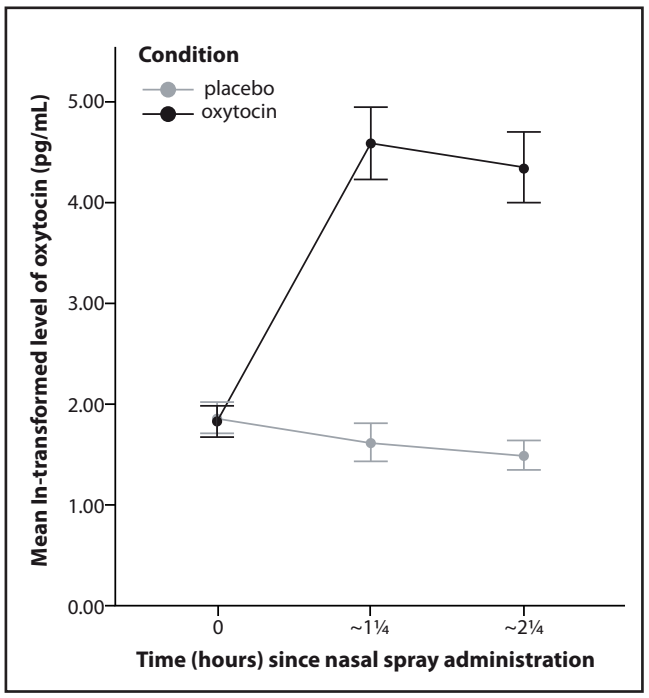

Fig. 1. Mean (ln-transformed) levels of salivary oxytocin before (T0), approximately 1¼ hours after (T1), and approximately 2¼ hours after (T2) administration of nasal spray containing 16 IU of oxytocin or a placebo. Vertical bars represent 95% confidence intervals.

DISCUSSION

Levels of salivary oxytocin increased markedly after intranasal oxytocin administration and remained elevated up to 2¼ hours after use of the nasal spray, whereas in the placebo condition (in which no oxytocin was administered) salivary oxytocin remained at a consistently low level. Within this 2 ¼-h period we also observed neurobehavioral effects of oxytocin. As reported elsewhere (Huffmeijer *et al.* 2011), oxytocin increased the amplitude of two ERP components, the vertex positive potential (VPP) and late positive potential (LPP), reflecting increased processing of and enhanced attention to the facial feedback stimuli. In the oxytocin condition we also found effects on altruism as indexed by donating money to a charity, in the study reported here (Van IJzendoorn *et al.* 2011). Several other studies conducted by our research group have also obtained effects of similar doses of oxytocin across a comparable time-range. Effects of oxytocin were, for example, found on neural responses (measured using fMRI) to infant crying (Riem *et al.* 2011) and infant laughter (Riem *et al.* in press) and on the use of excessive force when listening to infant crying (Bakermans-Kranenburg *et al.* 2011). Thus, the clear elevations of oxytocin levels in the current study were observed within a time-range in which its neurobehavioral effects are commonly observed, suggesting that salivary concentrations may be a valuable biomarker for oxytocin. Saliva samples are easily collected, for both participant and experimenter, and may routinely be collected during neurobehavioral experiments. Salivary levels of oxytocin may then be related to neurobehavioral outcome measures and (ultimately) add to the precision of the experimental effects.

During this first study investigating oxytocin in saliva after intranasal administration, salivary oxytocin was measured at only three time points at relatively large intervals (~1 h) for the duration of common neurobehavioral oxytocin experiments. To obtain a clearer idea of the time it takes for salivary levels of oxytocin to reach a maximum after intranasal oxytocin administration and to study the half-life of oxytocin in saliva, future studies should include a larger number of sampling times at shorter intervals. In addition, because salivary levels of oxytocin were still clearly elevated 2¼ h after use of the nasal spray, future studies may also include measurements of salivary oxytocin over a longer time-period. Furthermore, although neurobehavioral effects of intranasal oxytocin administration have been widely replicated, we do not know to what extent salivary oxytocin levels reliably reflect oxytocin levels in the brain. Considerable fluid mucus from the nasal passages is transported to the back of the throat and swallowed. Normally, a quart or more of fluid is generated daily, carried from the nose to the back of the throat and swallowed. However, some of the mucus does move from the throat into the mouth and mixes with saliva. Thus, some of the increase in salivary oxytocin following nasal administration may be from this direct movement of mucus; thus, the elevated levels in this study may not directly reflect elevated levels in either plasma or the brain. Studies relating salivary levels of oxytocin to both plasma oxytocin levels and to measures of brain oxytocinergic activity are important to shed light on this issue. In any case, the present study does confirm that a single nasal administration of oxytocin does not dissipate in seconds or minutes, but remains at high levels in saliva for more than 2 hours. This suggests a pattern that may parallel sublingual or transdermal administration of various medicines and other exogenous substances. Finally, all our participants were female, because of the considerable differences between males and females in the oxytocin system (Skuse & Gallagher 2009) and because of the dearth of experimental studies focusing on effects of oxytocin in females (see Van IJzendoorn & Bakermans-Kranenburg 2012). Future studies should focus on men as well.

In conclusion, we demonstrated elevated levels of salivary oxytocin up to 2¼ h after intranasal oxytocin administration, across a period in which neurobehavioral effects of oxytocin are commonly observed. Future studies are necessary to quantify rise times and half-life of oxytocin in saliva more precisely. Nevertheless, the current results indicate that salivary concentrations may be a valuable biomarker for oxytocin.

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