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Sedative Effects of Intranasal Oxytocin in Rabbits and Rhesus Monkeys

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Summary

Oxytocin is a hormone therapeutically used mainly for its peripheral effects during pregnancy in the uterus and breasts. However, additional central effects, i.e. anxiolytic effect, decreased level of social stress and increased empathy have been also observed. Hence, the aim of our study was to evaluate if nasal oxytocin can be used as anxiolytic substance in rhesus monkeys (n=20) and rabbits (n=20). Simultaneously, mean arterial blood pressure, arterial oxygen saturation of hemoglobin and pulse rate were monitored in all the evaluated animals. While rabbits lost righting reflex, monkeys developed a dose-dependent loss of aggressiveness and/or anxiety as evaluated by behavioral methods (aggressive behavior was classified as non-sedated – sedated – strongly sedated).

Key words

Oxytocin • Anxiety • Sedation • Monkeys • Rabbits

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Introduction

Oxytocin (CAS: 50–56–6) is a cyclic nanopeptide which acts as a hormone in all mammalian species. It is produced by neurohypophysis and binds to oxytocin receptors (OTR); however, it can also interact with arginine-vasopresine (AVP) receptors (Kimura *et al.* 1992). OTRs are primarily expressed in the uterus and mammary gland. Although these receptors have been shown to be expressed in other organs including the ovaries, mammary glands, testes, corpora cavernosa, prostate, adrenal glands, skin, osteoclasts, endothelial cells and adipose tissue, OTRs are also present in some brain areas (cortex, hippocampus, hypothalamus, olfactory bulb or striatum), thus suggesting neurotransmitter-like activity (Kimura *et al.* 1992, Knobloch and Grinevich 2014).

The fundamental mechanism of action of oxytocin is comprised of the contraction of smooth muscle cells in the uterus during pregnancy and milk release during lactation (Gimpl and Fahrenholz 2001). However, the presence of OTRs in other organs suggests that they are involved in additional complex interactions. The effects involve the modulation of the neuroendocrine reflexes and complex social and bonding behaviors. Some papers also demonstrate the modulation of stress in experimental animals (Guzman *et al.* 2014, Huang *et al.* 2014, Peters *et al.* 2014) and humans (Eckstein *et al.* 2014, Guzman *et al.* 2013, Heinrichs *et al.* 2003).

Since the behavioral effects of oxytocin in rodents are relatively homogenous, we decided to evaluate its effects in rabbits and rhesus monkeys. Additionally, we investigated if there were any detectable differences following intramuscular or intranasal administration.

The primary aim of our study was to confirm our hypothesis that oxytocin has sedative effect in different

animal orders (rabbits versus primates). The secondary aims of our study were to assess if the site of administration (intramuscular, resp. nasal) leads to greater effectiveness and has less side effects on the cardiorespiratory system.

Methods

Animals

Twenty (20) chinchilla rabbits, approximately 52 weeks of age (*Oryctolagus cuniculus*, Biotest Konárovice, Czech Republic), with a mean weight of 3.42 kg (range 2.5-4.5) from both sexes were used. The rabbits were housed in individual cages (70 × 60 × 40 cm), at room temperature (20-22 °C), relative humidity of 40-65 %, under in normal incandescent lighting (animals were exposed to lights between 6 A.M. to 6 P.M.). Food and water were available *ad libitum*. The rabbits received 5 IU of oxytocin via intranasal (n=10) or intramuscular (n=10) routes according to a randomization scheme.

Twenty (20) rhesus monkeys (*Macaca mulatta*, Biotest Konárovice, Czech Republic) with a mean weight of 4.10 kg (range 2.6-5.4), 1-3 years of age, from both sexes were used. The monkeys were housed in individual cages located in an outdoor enclosure. Food and water were available *ad libitum*, although access to food was restricted 12 h prior to the start of the experimental procedure; access to water was not restricted.

Experiments were approved by the Expert Committee for Protection of Experimental Animals of the Institute for Clinical and Experimental Medicine (IKEM) and were performed in accordance with the Animal Protection Act of the Czech Republic (No. 246/1992 Sb.).

Substances

For nasal administration, we used Oxytocin™ Ferring at a concentration of 5 IU/ml (equivalent to 8.5 μg in 1 ml). Oxytocin was provided as a nasal spray (IN) using the LMA©MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex, United States). The spray atomizes drugs into a fine mist of particles 30-100 μm in size. Intramuscular administration (IM) was performed by injections into the semitendinosus muscle (rabbits) or into the deltoid muscle (macaques). The dose was 5 IU (8.5 μg) in all groups, e.g. in average 2.5 μg/kg (1.5 IU/kg) in rabbits and 2.1 μg/kg (1.2 IU/kg) in macaques.

Procedures

Oxytocin was administered to rabbits after 15 min of acclimatization to the laboratory environment. Loss of righting reflex was used as a measure of onset of the sedative effect of oxytocin in rabbits. It was defined as the inability of a rabbit to right itself within 30 sec after placing on its back and was measured at 15 sec intervals. The interval between administration of the drug and the loss of righting reflex to occur was chosen for further statistical evaluation.

The arterial blood pressure (AP) and pulse rate (PR) were measured oscillometrically on the rabbits' forelimbs in 20 animals (10 readings from each group) (Memoprint S+B MedVet, Babenhausen, Germany). The sensor of the pulse oximeter (Nonin 80500 V, Plymouth, Minnesota, U.S.A.) was placed on the skin on the posterior part of the neck to measure the arterial oxygen saturation of hemoglobin (SpO_2) and PR. The baseline values were recorded before drug administration and thereafter, at corresponding time intervals (i.e. 1 min for SpO_2 and PR, 5 min for AP (the mean value of 3 readings taken over a 2 min time period) for 20 min.

Due to the naturally aggressive behavior of rhesus monkeys, oxytocin was administered either by nasal spray or muscular injection after the animal was temporarily restrained by a mechanic device integrated in the cage. After administration of the drug, the animals were closely observed for behavioral changes, i.e. sedation, ataxia, tactile reaction, loss of aggressiveness (reaction of the animal to an approaching hand or touch) and reaction to a sound stimulus (clap of the hands).

Aggressive behavior of the animals was classified as follows: 0 – non-sedated, actively observes its vicinity and tries to liberate itself; 1 – sedated, calm, partial loss of aggressiveness after slight touch by the investigator's finger; 2 – strongly sedated, calmly lying, full loss of aggressiveness, periods with closed eyes, unnecessary fixation was used for safety reasons only (Fig. 4 and 5). As soon as we were able to place the sensor of the pulse oximeter on the animals, we began measuring blood pressure, pulse rate, and oxygen saturation of hemoglobin (SO_2). The blood pressure and heart rate were measured oscillometrically on the monkey's forelimb (Memoprint S+B MedVet). The sensor of the pulse oximeter (Nonin 80500 V) was placed on a forelimb finger to measure SO_2 and heart rate. These measurements were taken every 5 min for up to 20 min.

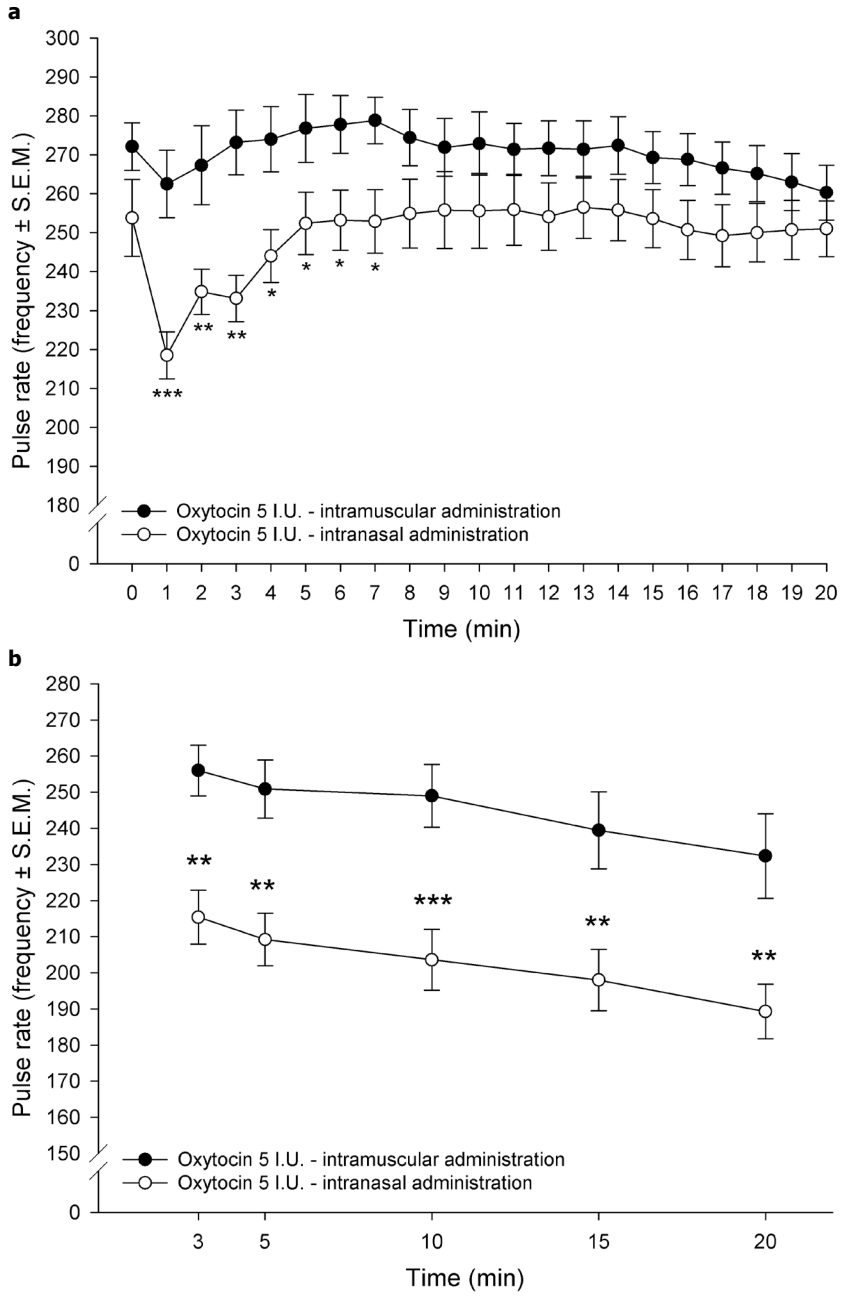

Fig. 1. Pulse rate in rabbits (**a**) (ANOVA, Tukey t-test; * p<0.037; ** p<0.01; *** p<0.001) and macaques (**b**) (ANOVA, Tukey t-test; ** p=0.002; *** p<0.001).

Statistical analysis

The cardiorespiratory parameters were evaluated using a two-way repeated measures ANOVA with one factor labelled "treatment" (intranasal vs. intramuscular) and the second factor labelled "time after administration" with a post-hoc Tukey t-test. The time to loss of righting reflex was evaluated using the Student's t-test. All statistical tests used two-tailed criterion, with a significance level of $p<0.05$. For the ANOVA tests, the F ratio (variance of the group means/mean of the within-group variance) with the corresponding degrees of freedom is presented.

Results

Rabbits

Loss of righting reflex

The intranasal administration of oxytocin resulted in the loss of righting reflex in six treated rabbits, while only two rabbits that received intramuscular administration exhibited the same result. The mean time (± standard deviation) to the loss of righting reflex was 225 (±107) and

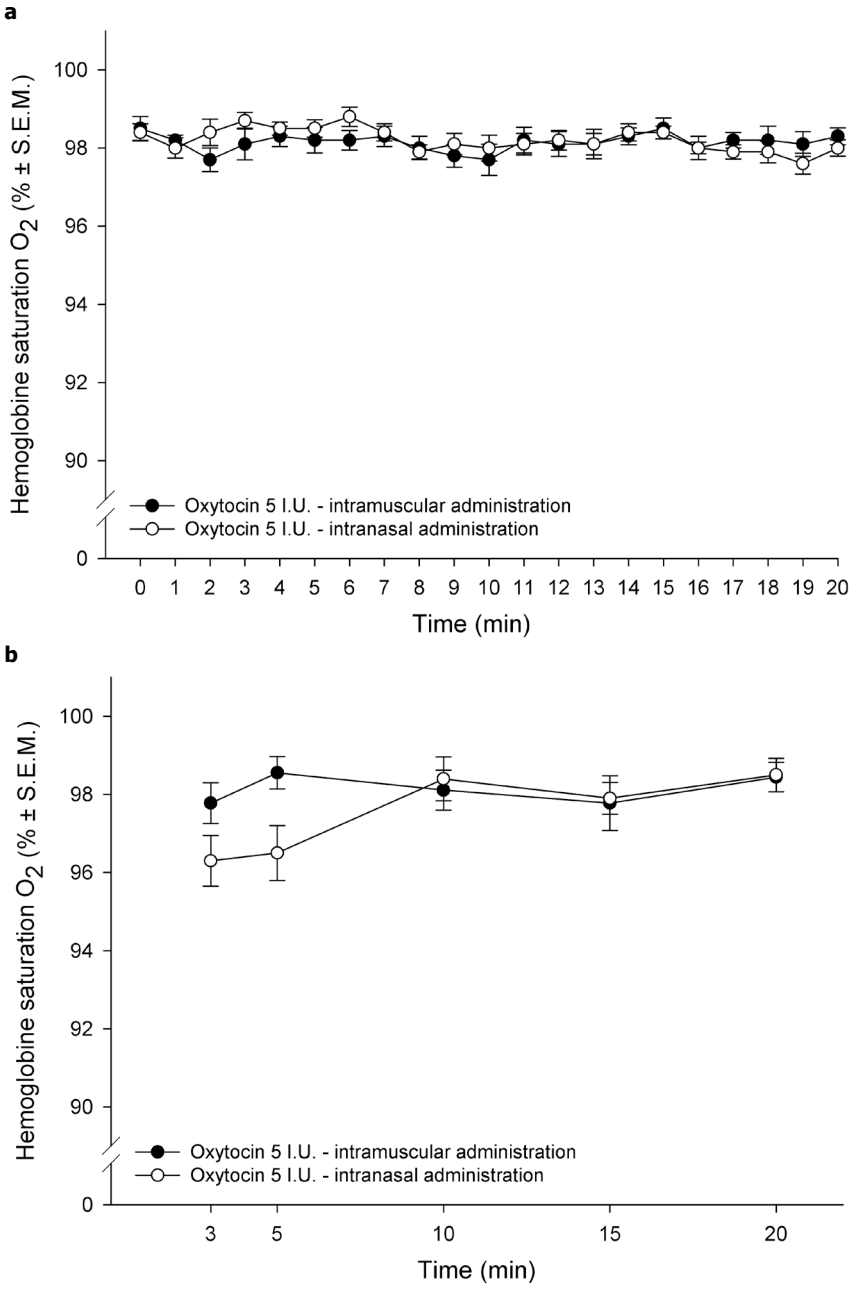

Fig. 2. Arterial oxygen saturation in rabbits (**a**) and macaques (**b**).

420 (±60) seconds after the administration of oxytocin intranasally and intramuscularly in those rabbits respectively, where a loss of righting reflex was achieved. The duration of effect minutes (±SD) was 15.3 (±2.6) and 15.5 (±5) after intranasal and intramuscular administration, respectively. The data were not statistically evaluated.

Pulse rate

The two-way repeated measures ANOVA, followed by the Tukey t-test, showed significant effects of treatment ($F_{1,419}=5.069$, $p=0.037$), time ($F_{20,419}=4.553$, $p<0.001$) and interaction of treatment × time ($F_{20,419}=2.415$, $p<0.001$) on PR (Fig. 1a). In general, the intranasal administration of oxytocin significantly reduced PR when compared to intramuscular administration ($p=0.037$) with statistically significant differences between these two types of treatment after 1-7 min (Fig. 1a).

Arterial oxygen saturation of hemoglobin

The two-way repeated ANOVA showed no significant effect between the two types of treatment on the SpO_2 ($F_{1,419}=0.0894$, $p=0.768$, Fig. 2a).

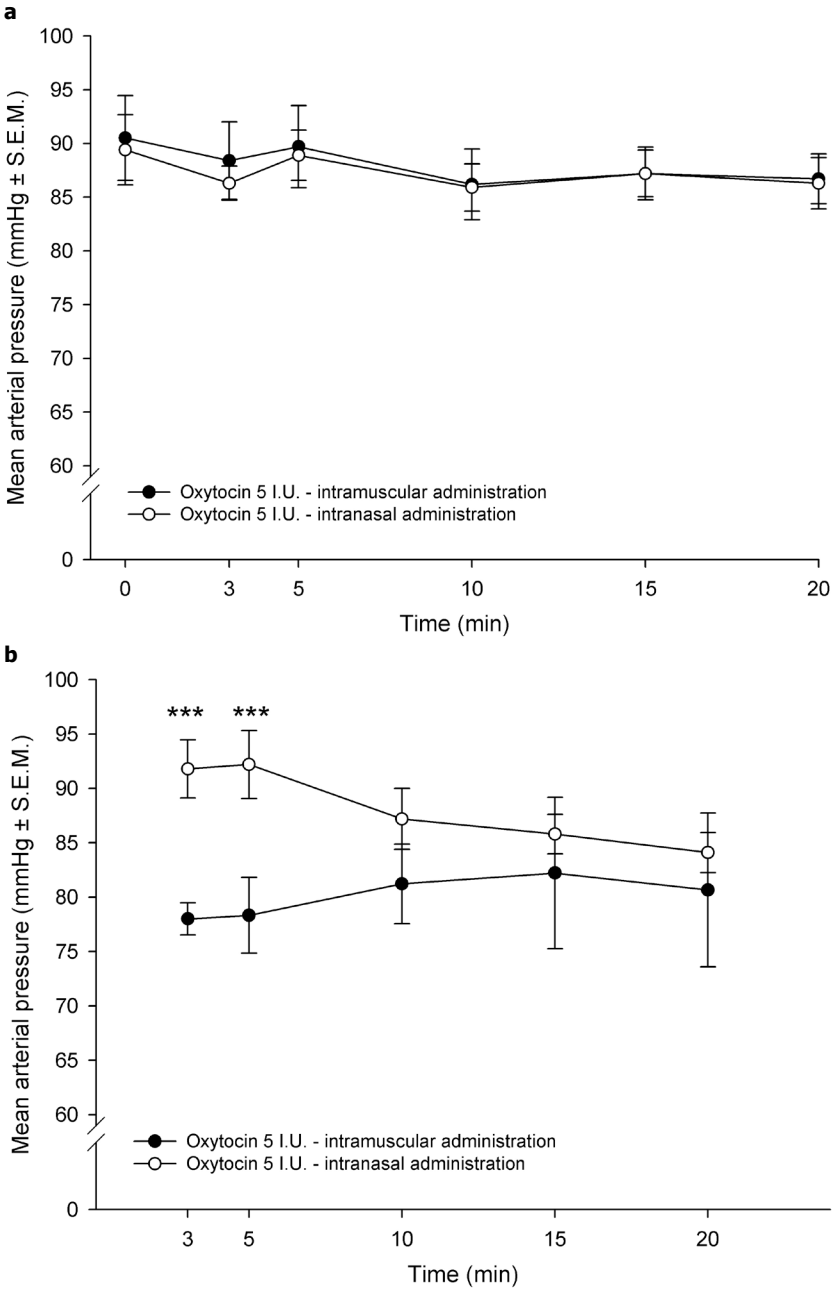

Fig. 3. Mean arterial pressure in rabbits (**a**) and macaques (**b**) (ANOVA, Tukey t-test; *** p=0.015).

Mean arterial blood pressure

The two-way repeated ANOVA showed no significant effect between the two types of treatment on the systolic mean arterial blood pressure ($F_{1,119}=0.0912$, $p=0.766$, Fig. 3a).

Macaques

Loss of aggressive behavior

The intranasal administration of oxytocin resulted in a rapid onset of sedation in most of the evaluated animals. Each animal developed early signs of sedation (eye narrowing, head declined, muscle relaxation) within several minutes after administration. Seven animals exhibited a score of 1 and 13 animals exhibited a score of 2. All of them easily tolerated touching of the face (mouth and nose), hence exhibiting a loss of aggressiveness (Fig. 4). Calm lying on the table without restraint is shown in Figure 5. Importantly, there was no substantial difference between the nasal and intramuscular route of administration in time to immobilization and time to loss of gripping reflex.

Fig. 4. Easily tolerated finger touch.

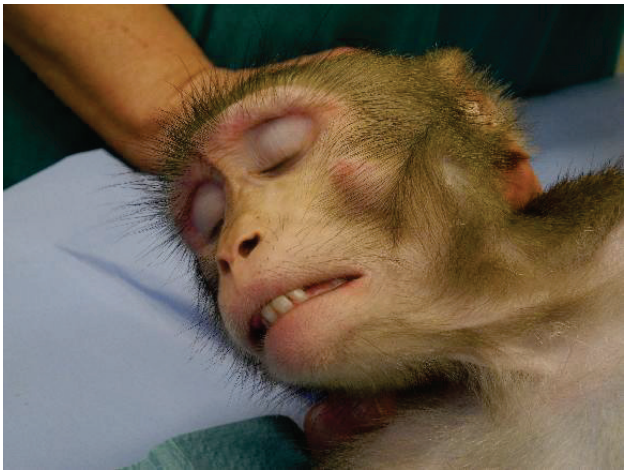

Fig. 5. Calm lying on the table.

Pulse rate

The two-way repeated measures ANOVA, followed by the Tukey t-test, showed significant effects of treatment ($F_{1,94}=14.575$, $p=0.001$) and time ($F_{7,94}=12.272$, $p<0.001$) on PR. Intranasal administration of oxytocin significantly reduced PR when compared to intramuscular administration ($p=0.002$) with a statistically significant difference between those two types of treatment in all intervals measured (Fig. 1b).

Arterial oxygen saturation of hemoglobin

The two-way repeated ANOVA showed no significant effect between the two types of treatment on arterial oxygen saturation of hemoglobin ($F_{1,94}=1.386$, $p=0.255$, Fig. 2b).

Mean arterial blood pressure

The two-way repeated ANOVA, followed by the Tukey t-test, showed significant effects of treatment ($F_{1,94}=7.399$, $p=0.015$) and the interaction of treatment × time ($F_{4,94}=4.312$, $p=0.004$) on the mean arterial blood pressure. Intramuscular administration of oxytocin significantly reduced mean arterial blood pressure compared to intranasal administration ($p=0.015$) with a statistically significant difference between those two types of treatment after 3 and 5 min after treatment (Fig. 3b).

Discussion

We found no studies concerning oxytocin in rabbits and studies evaluating effect of nasal oxytocin in monkeys are rare with conflicting results. Moreover, there is no available data on using oxytocin for sedation and/or loss of aggressiveness. We confirmed our hypothesis that oxytocin produces mild sedation. Although oxytocin is not a typical sedative agent, our results clearly demonstrated the sedative effects of oxytocin administered either intranasally or intramuscularly in two evolutionary different animal orders, rabbits and macaque monkeys. Knobloch *et al.* (2012) relatively recently described the association between axonal projections of hypothalamic oxytocin neurons in the majority of forebrain regions, including the amygdala, a structure which plays an important role in oxytocin-mediated fear suppression. He also notes that oxytocin release these by neuronal endings may specifically control region-associated behaviors.

As an example, oxytocin was found to mediate mating-induced anxiolysis in male rats as it substantially increases risk-taking behavior (Waldherr and Neumann 2007). Of importance, oxytocin administration was previously shown to significantly decrease anxiety in the four-plate test, elevated zero maze, and stress-induced hyperthermia in mice. Hence, the authors dispute its possible utility as an anxiolytic drug in humans (Ring *et al.* 2006).

On the other hand there are many questions unanswered. The second major result of our study, with the exception of confirming our hypothesis, is the different effects of oxytocin between the two different animal orders – rabbits and primates. Rabbits developed a loss of righting reflex within several minutes in a limited number of individuals only. Compared to rabbits, all monkeys, in spite of a moderately lower dose per kg, were substantially sedated as expressed by loss of aggressiveness, i.e. they lied calmly on the table and allowed touching of the face close to the mouth and nose.

The explanation is not easy. We can speculate that either primates are more sensitive to the effect of oxytocin or the tools we used for measurement of effect of oxytocin were more sensitive in primates than in rabbits. The first possibility can be supported by several studies in humans, where a low dose of nasal oxytocin (12-24 IU, i.e. 0.2-0.4 IU/kg) can induce subtle changes in behavior (MacDonald *et al.* 2011, MacDonald and Feifel 2014). Parr *et al.* (2013) used 48 IU oxytocin administered by a nebulizer and found that macaques had selectively reduced attention to negative facial expressions, but not neutral social or non-social images, and had no signs of sedation. The second explanation may be that a loss of righting reflex in rabbits may not be sensitive enough to detect the discreet changes of behavior like the loss of aggressiveness in primates. The solution needs further research, mostly because the majority of research in experimental animals was performed on rats and mice (MacDonald and Feifel 2014). If interspecies differences exists, it must be considered for further research and while planning experiments.

The other discrepancy is in regards to the onset of effect, which was quite short in our study compared to other studies. Though, the majority of studies concentrated on measurement of oxytocin levels in various body compartments and not to its demonstrable effects. Neumann *et al.* (2013) measured the concentration of oxytocin by micro-dialysis in the amygdala and hippocampus in rats and mice and demonstrated that after nasal administration of oxytocin, the peak level was 30-60 min from the time of delivery. Modi *et al.* (2014) used a much higher dose than in our experiment and examined the cerebrospinal fluid concentration of oxytocin in macaques after intravenous administration, intranasal spray and aerosolized intranasal administration by a mask. All three administration routes elevated plasma concentrations, but only the aerosolized route significantly increased concentrations in cerebrospinal fluid, which could be important factor for the final effect. In our study, we were able to induce sedation and loss of aggressiveness by using only 5 IU. The reason may be that cerebrospinal fluid concentration does not reflect behavioral changes, which were not measured in Modi's study. Gossen *at al.* (2012) measured plasma levels after nasal administration of oxytocin in humans and demonstrated peak levels after 30 min with return to baseline after 90-150 min. Again, the plasma levels does not necessarily reflect effects on central nervous system (Gossen *et al.* 2012).

Of particular importance, the administration of oxytocin had no influence either on mean arterial pressure or hemoglobin saturation. The initially recorded difference in arterial pressure in macaques at the beginning of experiment can be easily explained by the manipulation of the animals, i.e. when administered intranasally, the animals had to be trapped – hence resulting in increased stress on the animal, which leads to higher blood pressure measurements. On the other hand, no such manipulation was needed in animals undergoing intramuscular administration.

Conclusions

In conclusion, our study brings new insight on the CNS effect of oxytocin. The interpretation of our results warrants further clinical research concentrated on anxiolytic and sedative effects in humans. In this study, oxytocin exhibited an absence of serious adverse effects, effectiveness even in low doses and a non-invasive method of administration.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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