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SHORT COMMUNICATION

Intranasal oxytocin administration attenuates the ACTH stress response in monkeys

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Summary Social relationships protect against the development of stress-related psychiatric disorders, yet little is known about the neurobiology that regulates this phenomenon. Recent evidence suggests that oxytocin (OT), a neuropeptide involved in social bond formation, may play a role. This experiment investigated the effects of chronic intranasal OT administration on acute stress-induced hypothalamic-pituitary-adrenal (HPA) axis activation in adult female squirrel monkeys. Subjects were randomized to one of two experimental conditions. Monkeys were intranasally administered either 50 μg oxytocin ($N=6$ monkeys) or 0 μg oxytocin ($N=6$ monkeys)/300 μl saline once a day for eight consecutive days. Immediately after drug administration on the eighth day, all monkeys were exposed to acute social isolation. Blood samples for determinations of adrenocorticotropic hormone (ACTH) and cortisol concentrations were collected after 30 and 90 min of stress exposure. Consistent with an anti-stress effect, OT-treated monkeys exhibited lower ACTH concentrations compared to saline-treated monkeys after 90 min of social isolation ($F_{1,7}=6.891$; $P=0.034$). No drug-related differences in cortisol levels were observed, indicating that OT does not directly attenuate the adrenal stress response. Intranasal peptide administration has been shown to penetrate the central nervous system, and research must determine whether intranasally delivered OT exerts its effect(s) at a pituitary and/or brain level. This primate model offers critical opportunities to improve our understanding of the anti-stress effects of OT and may lead to novel pharmacological treatments for stress-related psychiatric disorders.

1. Introduction

It is well established that stressful events contribute to the development of depressive and anxiety disorders (Brown and Harris, 1993; Paykel, 1978). In mammals, the stress response is primarily mediated

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through the hypothalamic–pituitary–adrenal (HPA) axis. Of all the biological systems examined in the pathogenesis of depressive and anxiety disorders, HPA axis abnormalities have been most consistently implicated (Arborelius et al., 1999; Holsboer, 2001; Parker et al., 2003).

In contrast to stressful events, strong social relationships provide important health benefits. Social support decreases stress responsivity, protects against the onset of psychiatric disorders, and improves treatment outcomes in patient populations (Paykel, 2001). Little, however, is known about the biological mechanisms that regulate this phenomenon. A systematic analysis of this underlying biology is warranted and may have important clinical implications. We investigate herein a role for the neuropeptide, oxytocin (OT), which exhibits promising anti-stress properties (Amico et al., 2004; Heinrichs et al., 2003; Neumann, 2002; Windle et al., 2004).

OT is synthesized in the hypothalamus and released into systemic circulation via the posterior pituitary. OT receptors are found in many peripheral sites, including the pituitary and adrenal glands (Gimpl and Fahrenholz, 2001). OT is also released into the central nervous system via widely distributed pathways, and central OT receptors are found in a variety of socially relevant and stress-sensitive limbic brain regions (Gimpl and Fahrenholz, 2001). Central OT administered to rodents facilitates social contact, maternal–infant attachment, and pair-bond formation (Pedersen et al., 1992; Williams et al., 1992; Witt et al., 1992). In humans, intravenously administered OT attenuates metyrapone-induced ACTH secretion (Chiodera and Coiro, 1987). Studies of rats corroborate this latter finding, as centrally administered OT diminishes stress-induced corticotropin-releasing factor (CRF) mRNA expression in the hypothalamus and attenuates the neuroendocrine stress response (Windle et al., 2004). OT deficient mice, in contrast, exhibit greater stress-induced corticosterone responses (Amico et al., 2004). This collective evidence suggests the intriguing hypothesis that OT released during positive social interactions reduces the HPA axis response to stressful events (Uvnas-Moberg, 1998), and in humans, may account for the extensive evidence that social support protects against the onset of stress-related depressive and anxiety disorders.

Despite these promising findings, OT has little potential as a therapeutic agent if its route of administration is invasive (e.g. central; intravenous). These invasive methods have also curtailed OT research in species other than rodents, and as a result, few studies have directly assessed the anti-stress effects of OT in monkeys and humans. An alternative to these invasive techniques is intranasal administration, which presents a viable, non-invasive delivery method (Liu et al., 2001). Moreover, peptides administered intranasally are delivered directly to cerebrospinal fluid in humans (Born et al., 2002) and a variety of brain regions in rats (Ross et al., 2004) and squirrel monkeys (Balin et al., 1986). In a recent experiment, a single intranasal OT administration, combined with social support, was found to decrease salivary cortisol responses to psychological stress in humans (Heinrichs et al., 2003). In this monkey experiment, we extend this initial research to investigate the effects of chronic intranasal OT administration—without the added buffer of social support—on stress-induced increases in plasma adrenocorticotropin hormone (ACTH) and cortisol.

2. Methods

2.1. Subjects

Twelve adult female squirrel monkeys (age range 6–17 years) of Guyanese origin (*Samiri sciureus*) served as subjects in this experiment. Monkeys were housed in same-sex social groups at the Stanford Research Animal Facility in $1.8 \times 1.2 \times 1.8$ m wire-mesh cages that were cleaned daily. Housing and testing occurred in climate-controlled rooms on a 12:12 light/dark cycle with an ambient temperature of 26 °C. Monkeys had ad libitum access to water, food (e.g. commercial New World monkey chow, fresh fruits, vegetables), and a variety of toys to provide environmental enrichment. A sliding door in each home cage facilitated access to a small, portable capture cage. Monkeys were trained to enter the capture cage on vocal command to facilitate experimental manipulations. All procedures were approved by Stanford University's Administrative Panel on Laboratory Animal Care.

2.2. Intranasal administration

Pilot studies to confirm intranasal efficacy and appropriate fluid volume for monkeys were conducted prior to the beginning of this experiment. Monkeys were then extensively acclimated to the intranasal procedure. This technique involved two experimenters and consisted of one experimenter (C.L.B.) swaddling the conscious monkey and gently restraining it in a supine position. A second experimenter (K.J.P.) swabbed each nostril to remove any mucous obstructing the nasal cavity.

Thereafter, the second experimenter administered a total volume of 300 μl of physiological saline (Abbott laboratories, Chicago, IL) using a pipette. A 50 μl volume was administered to each nostril on an alternating basis, with a 30 s rest period in between each nostril administration. Each 50 μl volume was delivered in a series of 3–4 μl drops, with each drop administered as the monkey inhaled. Monkeys occasionally sneezed, and a volume equivalent to the estimated expelled volume was re-administered. After delivery of the 300 μl volume, monkeys were maintained in a supine position for an additional minute to increase fluid uptake. The intranasal delivery method took 12.8±0.5 min to complete.

2.3. Experimental design

The oxytocin compound (98% pure as determined by HPLC) used in this experiment was generously supplied to us by Professor Maurice Manning of the Medical College of Ohio. Determination of the oxytocin dose was based on the human literature (Bruins et al., 1992; Epperson et al., 1996; Heinrichs et al., 2003) and pilot data, which indicated that chronically administered OT at high doses (e.g. 200 μg) did not diminish stress responsivity. OT, and the closely related neuropeptide, arginine-vasopressin (AVP), can exert physiological effects by binding to each other's receptors (Cho et al., 1999; Engelmann et al., 1996), particularly at higher concentrations. Because AVP potentiates the stress response (Legros, 2001), partial occupancy of AVP receptors following high dose OT administration may offset the anti-stress effects of OT. We thus chose a lower OT dose for use in this experiment to guard against this possibility.

Monkeys were randomized to one of two experimental conditions, in which the aforementioned intranasal administration procedure was used. In one condition, monkeys were administered 50 μg (~23 IU) of oxytocin/300 μl saline. In the other condition, control monkeys were administered 0 μg oxytocin/300 μl saline. Monkeys received intranasal administrations once a day for eight consecutive days. Immediately following the eighth intranasal administration, monkeys were placed alone in a cage (60×60×90 cm) for 90 min. Acute social isolation is a reliable and well-established method by which HPA axis activation is elicited in adult squirrel monkeys (Lyons et al., 1999). At the end of the 90 min period, each monkey was returned to the home cage and housed under standard laboratory conditions.

2.4. Blood collection and hormone assays

Blood samples were collected two weeks before the acclimation period to establish baseline measures of ACTH and cortisol in an undisturbed state. Subsequently, blood samples were collected 30 and 90 min after the beginning of stress exposure to examine the effects of chronic oxytocin administration on the HPA axis stress response. Blood samples were collected between 1430 and 1830 h to control for circadian variation in hormone levels (Zeitzer et al., 2003).

Blood samples were collected from manually restrained monkeys while blood (0.8 ml) was drawn by femoral venipuncture with single-use syringes containing 20 μl of ethylenediamine tetraacetic acid (EDTA). Each blood sample was immediately centrifuged and the plasma fraction was stored at −80 °C. Hormones were measured in duplicate using commercially prepared ACTH (Diasorin, Inc., Stillwater, MN) and cortisol (Diagnostic Products Corporation, Los Angeles, CA) radioimmunoassays as previously described (Lyons et al., 1995; Parker et al., 2004). The intra- and inter-assay coefficients of variation for ACTH were 5 and 8%, and 10 and 13%, respectively, for cortisol. Assay sensitivity was 9 pg/ml for ACTH and 4 μg/dl for cortisol.

2.5. Data analysis

The effect of intranasal OT administration on stress-induced increases in ACTH and cortisol levels was assessed with repeated measures analysis of variance (ANOVA) using least squares estimates from general linear models in the MGLH module of Systat, Inc. (Point Richmond, CA). Drug treatment was considered a between subjects factor and blood collection time point was considered the repeated measures within subjects factor. Intranasal administration latency and the number of re-administered drops on the day of blood sampling were used as statistical covariates, as was each subject's age. For all analyses, test statistics were evaluated with two-tail probabilities ($P<0.05$).

3. Results

Most blood samples (93%) were collected within 180 s from cage entry (median latency to sample collection, 46 s; range 24–242 s), and all but one sample (99%) was collected within 240 s. In keeping with reports that squirrel monkey plasma measures of ACTH and cortisol obtained within these time limits and using these procedures do not reflect

disturbance effects from sampling per se (Lyons et al., 1995, 1999), sample collection latencies accounted for less than 1% of the variance in plasma levels of both ACTH and cortisol.

No differences in basal ACTH or cortisol levels were found between treatment groups prior to the beginning of the study. In keeping with the notion that OT attenuates the HPA axis stress response, a drug×time point interaction was observed ($F_{2,14}=4.769$; $P=0.026$). Specifically, OT-treated monkeys exhibited diminished ACTH levels compared to vehicle-treated monkeys after 90 min of stress exposure ($F_{1,7}=6.891$; $P=0.034$). No drug-related differences in stress-induced cortisol levels were observed (Fig. 1).

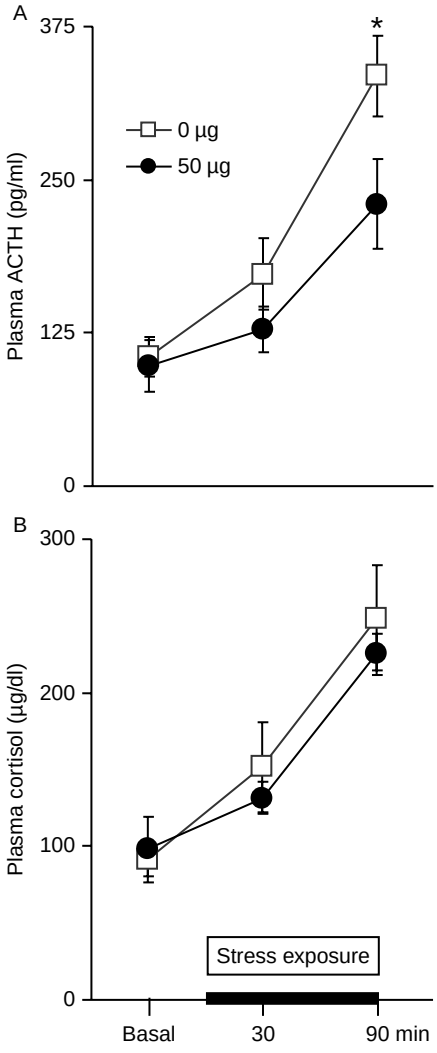

Figure 1 Intranasal administration of oxytocin attenuates the ACTH stress response. Plasma measures of (A) ACTH and (B) cortisol at baseline and 30 and 90 min after the beginning of stress exposure are presented for adult female squirrel monkeys treated with 50 µg oxytocin/300 µl saline ($N=6$) or 0 µg of oxytocin/300 µl saline ($N=6$). Data are presented as mean±SEM. Asterisks indicate time points during which oxytocin-treated and saline-treated monkeys differed ($P<0.05$).

4. Discussion

This experiment demonstrated that chronic intranasal oxytocin administration prior to acute social isolation attenuates the ACTH response to stress in monkeys. No significant effect of OT on stress-induced cortisol levels was observed. Because the adrenal response to stress temporally follows that of the pituitary, it is likely that our assessment period was too short to capture the treatment-related changes in cortisol levels reported by others (Neumann, 2002; Windle et al., 2004). It has been previously hypothesized that intravenously administered OT exerts its effects directly at the adrenal gland by altering cortisol synthesis and/or release (Legros et al., 1988). Evidence from our study, however, suggests that intranasally administered OT does not directly alter the stress response at an adrenal level, but rather exerts its anti-stress effects indirectly, prior to adrenal activation. Although a peripheral effect of OT cannot be ruled out, intranasal peptide administration has been shown to penetrate the squirrel monkey central nervous system (Balin et al., 1986). Future research must now determine whether OT exerts its anti-stress effect(s) at a pituitary and/or brain level.

Many other aspects of the effects of OT on primate stress physiology remain to be characterized. In our pilot studies, for instance, a single intranasal OT administration before exposure to a more potent stressor than social isolation (i.e. chair restraint) did not attenuate the ACTH stress response (unpublished data). A single intranasal OT administration in humans was only effective in diminishing stress responsivity when combined with social support, as the anti-stress effects of OT alone failed to reach statistical significance. Whether these findings are a function of a stressor's severity, or whether chronic OT administration more strongly inhibits primate HPA axis activation, as documented for rodents (Petersson et al., 1999), warrants investigation.

One final aspect of this study that merits consideration is the possibility that the anti-stress effects of OT were influenced by the reproductive state of our subjects. Squirrel monkeys are seasonal breeders, and this study was conducted during the breeding season when ovarian steroid hormone levels, particularly estrogen, are elevated relative

to the non-breeding season (Mendoza et al., 1978). Estrogen has been shown to enhance the effects of OT-mediated social behavior, increase oxytocin receptor densities in socially relevant and stress-sensitive brain regions, and attenuate the HPA axis stress response (Witt, 1997; Young et al., 2001). Although it is unclear whether the ovarian cycle, per se, influences the HPA axis response to psychological stressors, the role of fluctuating ovarian hormone levels and their possible role in modulating the anti-stress effects of OT should be directly addressed in future research endeavors.

In summary, this study provides initial evidence that chronic intranasal OT administration diminishes the ACTH stress response in primates. These findings support the theory that OT attenuates HPA axis activation, and, in humans, may protect against the development of stress-related psychiatric disorders. This primate model offers critical opportunities to improve our understanding of the anti-stress effects of OT, and will allow us to research aspects of physiology that are difficult to study in humans (e.g. repeated blood and cerebrospinal fluid sampling). With continued investigation, this research may one day lead to novel pharmacological treatments for stress-related psychiatric disorders.

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