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Plasma oxytocin in response to pharmaco-challenge to D-fenfluramine and placebo in healthy men

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Abstract

The neuropeptide oxytocin is produced in the hypothalamus and released centrally and peripherally in response to serotonergic stimulation. Plasma oxytocin levels may be a candidate as a biological index of serotonergic function in response to pharmacological challenge by serotonergic agents. Fourteen male healthy subjects underwent a placebo challenge and a D-fenfluramine (D-FEN) (0.5 mg/kg) challenge on different days. Serial plasma oxytocin and prolactin levels were measured on each challenge day. D-FEN was associated with an increase in both oxytocin and prolactin. Plasma oxytocin may function as an index of central serotonin (5-HT) function in human subjects. Since oxytocin is released directly from limbic-hypothalamic cells, this response presumably represents a direct central assessment of 5-HT response in limbic-hypothalamus. Further work will determine if the oxytocin response to 5-HT pharmaco-challenge, by virtue of its central origin, is more highly related to measures of psychopathology (e.g. aggression) than that of less central outcome parameters of 5-HT responsiveness (e.g. prolactin).

Keywords: Prolactin; Serotonin; 5-HT; Neurohormone; Pharmacochallenge; Hypothalamus; Vasopressin

1. Introduction

The study of the brain serotonin (5-HT) system in human subjects includes, among other methodologies (e.g. cerebrospinal fluid metabolites), assessment of central synaptic response through the measurement of a behavioral, thermal, or hormonal response to acute 5-HT challenge (Coccaro et al., 1995). Of these three broad outcome variables, hormonal responses are thought to be less direct and less central in nature because there is at least one intermediate step between the 5-HT synapse in question and the manifest hormonal response (e.g. in the case of prolactin and adrenocorticotropic hormone, this step is represented by the production of a prolactin- or a corticotropin-releasing factor) and because nearly all hormonal

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responses used in 5-HT challenge studies originate outside the brain (e.g. anterior pituitary in the case of prolactin and adrenocorticotropic hormone). While hormones directly released by central structures would provide a more direct and central window into synaptic functioning, few such candidates have been studied to date.

One potential central hormone candidate is oxytocin. Oxytocin is produced in the lateral magnocellular neurons of hypothalamic paraventricular and supraoptic nuclei. While 5-HT nerve fibers are relatively scarce in the paraventricular nucleus, 5-HT terminals are concentrated in regions with oxytocinergic neurons (Chiodera et al., 1994). Stimulation of the hypothalamus by serotonin leads to release of oxytocin as a precursor molecule. The precursor is sent via neurosecretory granules down axons of the neurohypophysis. During exoplasmic transport, the carrier molecule is then cleaved from the neurohormone at which point oxytocin is released into capillary space in posterior pituitary by depolarization and exocytosis. Unlike other neurohormones such as prolactin or adrenocorticotropic hormone that require a 'releasing factor', there is no intermediary peptide in the pathway (Cleare et al., 1998). In addition, oxytocin neurons are capable of releasing oxytocin from somata and dendrites into the extracellular fluid of their nuclei of origin. Central intrahypothalamic release of oxytocin from somata and dendrites is independent from, but possibly coupled to, secretion into the bloodstream from the pituitary (Neumann et al., 1993).

Evidence in animals and in humans suggests that serotonergic challenge agents lead to measurable changes in plasma oxytocin concentration. Animal studies demonstrate that 5-HT receptor stimulation by 5-HT_{1A} and 5-$HT_{2A/C}$ agonists induces an increase in plasma oxytocin levels (Saydoff et al., 1991; Li et al., 1993a,b; Bagdy et al., 1992; Van de Kar et al., 2001). In contrast, results from human studies have shown more variability. While low doses of the 5-HT_{1A} agonist ipsapirone (20 mg) were reported to stimulate release of plasma oxytocin in male and female volunteers (Cleare et al., 1998), a placebo-controlled study showed no significant effect on plasma oxytocin at 0.3 mg/kg in healthy male and female volunteers (Newman et al., 1999). Using *m*-chlorophenylpiperazine (*m*-CPP), a 5-HT agonist with 5-HT_{2C} selectivity, Bagdy and Arato (1998) found a gender-dependent effect, with *m*-CPP challenge leading to a measurable oxytocin release in female, but not male, healthy human volunteers.

D-Fenfluramine (D-FEN) is a 5-HT probe with multiple 5-HT receptor effects (including those at the 5-HT_2 receptor) that has not been studied in humans with respect to oxytocin response. While D-FEN is no longer available for studies in human subjects, it has the theoretical advantage of being an agent that acts on multiple 5-HT receptor subtypes, including 5-HT_{1A} and 5-HT_2 receptors. Accordingly, the purpose of this study was to determine if pharmacochallenge with D-FEN results in a central oxytocin response measurable in the plasma and if an oxytocin response to D-FEN would correlate with the more established prolactin response to D-FEN (PRL [D-FEN]).

2. Methods

Subjects were 14 physically healthy male volunteers recruited by advertisement to participate in studies of endocrine response to D-FEN challenge as approved by the IRB of the MCP Hahnemann School of Medicine. Physical health of the subjects was evaluated by medical history, physical examination, standard laboratory studies (hematology, chemistries, thyroid function, urine drug screen, etc.) and electrocardiogram, and mental health was evaluated by a research examination designed to determine the presence of Axis I and II disorder. Only subjects who were medically healthy, without any Axis I/II disorder and without a family history of any treated major psychiatric disorder were studied. Written informed consent, using an IRB-approved consent document, was obtained from all subjects after the procedures were fully explained. Subjects who qualified for the study and who provided written informed consent, underwent a placebo challenge and a D-FEN (0.5 mg/kg) challenge on different days (mean interval: 8.1 ± 5.5 days) in which the sequence was randomized. The mean age of subjects was 31.6 ± 8.9

years, and no subject had a history of psychopharmacological treatment of any kind.

2.1. Fenfluramine challenges

Subjects were at least 2 weeks free of any medication of any kind. Subjects also were instructed to follow a low monoamine diet for 3 days prior to each test session. All subjects came to the Clinical Neurosciences Research Unit Procedures Suite at approximately 08:00 h after an overnight fast. At approximately 08:30 h, an intravenous catheter (IV) was inserted in a forearm vein and kept open by a slow saline drip. The IV catheter was placed for the purpose of repeated venopuncture before and after administration of D-FEN or placebo. Basal blood samples for PRL and OXY were obtained at 09:45 h and 09:55 h. The sample was immediately transferred to a tube containing EDTA and centrifuged at 4 °C. Plasma was then transferred to two polypropylene tubes. Both tubes were frozen immediately on dry ice and then stored at either −70 °C (OXY) or at −20 °C (PRL). D-FEN (0.5 mg/kg) or placebo was given orally at 10:00 h. Post-FEN blood samples were obtained every 30 min for a total of 5 h (10:00 h through 15:00 h). These data included measurements of blood pressure, temperature, and subjective emotional state (Visual Analogue Scale).

2.2. Diagnostic assessment

Axis I and Axis II diagnoses were made according to DSM-IV criteria with final diagnoses being assigned by best-estimate consensus procedures as previously described (Coccaro et al., 1996a,b,c,d). Diagnoses were made using information from: (a) semi-structured interviews by trained clinicians using the Schedule for Affective Disorders and Schizophrenia (Spitzer and Endicott, 1978) or the Structured Clinical Interview for DSM Diagnoses (Spitzer et al., 1992) and the Structured interview for the Diagnosis of DSM Personality Disorder (Pfohl and Zimmerman, 1989).

2.3. Assays for plasma oxytocin and plasma prolactin

Plasma oxytocin (OXY) was measured by a method modified from Keil et al. (1984). The sensitivity limit of the assay is 1 pg/tube, and the intra-assay coefficient of variation (CV) is 8.1% while the inter-assay CV is 8.5%. Plasma prolactin (PRL) was assayed by radioimmunoassay (RIA, Diagnostic Products Corporation, Los Angeles, CA). The prolactin RIA sensitivity is 1.5 ng/ml with a working range of 2.5–200 ng/ml. The intra-assay CV is approximately 4%, while the inter-assay CV is 6%. Samples were in frozen storage for a mean of 58.9 months before being assayed. The minimum storage time was 14 months. The maximum storage time was 72 months. Time to assay was not significantly correlated with peak plasma oxytocin concentration ($r=0.334$, $N=14$, $P=0.243$). A positive correlation for sample storage time and plasma oxytocin area under the curve (AUC) was found at the trend level ($r=0.587$, $N=14$, $P=0.064$), arguing against sample decay.

2.4. Statistical analysis

Repeated-measures of analysis of variance (RM-ANOVA) with condition (placebo vs. D-FEN) and time (−15 min through +300 min) was used to analyze plasma hormone levels. For analyses involving peak delta PRL [FEN] and OXY [FEN], hormonal levels taken before D-FEN challenge at −15 min and −5 min were averaged to determine a single baseline value to use with post-fenfluramine hormonal levels to calculate a peak delta hormonal value ('peak post-FEN value' minus 'averaged baseline value'). Additional comparisons were performed after measuring area under the curve for oxytocin and prolactin response to placebo and D-FEN. AUC values for PRL [D-FEN] and OXY [D-FEN] were estimated by the trapezoid rule. Correlations were performed by Pearson's correlation. All alpha (0.05) probability values reported are two-tailed.

3. Results

3.1. D-Fenfluramine dosage

The mean D-FEN dose administered was 40.71 mg. The maximum was 50 mg, the minimum was 30 mg, and the standard deviation was ±5.5 mg.

3.2. Baseline plasma oxytocin and prolactin

Baseline values for oxytocin on placebo and D-FEN challenge days were highly correlated ($r=0.83$, $P=0.01$), as were the baseline values for prolactin on placebo and D-FEN challenge days ($r=0.84$, $P=0.01$). No significant differences were found for either basal or peak delta oxytocin or prolactin response between those who received D-FEN first or second.

3.3. Plasma oxytocin responses to D-FEN and placebo (Fig. 1)

To determine the effect of fenfluramine administration compared to placebo on plasma oxytocin concentration, data were subjected to a 2 (group status)×12 (time) RM-ANOVA. For oxytocin, RM-ANOVA revealed a significant effect for time (F (5, 65)=4.55, $P<0.001$) and for the drug×time interaction (F (5, 65)=6.15, $P<0.001$). Main effect for the condition (placebo vs. D-FEN) approached, but did not reach, statistical significance (F (1, 13)=3.49, $P=0.084$). Additionally, significant differences were found for area under the curve between oxytocin response to D-FEN ($M=343.12$, S.D.=334.12) and placebo ($M=-83.41$, S.D.=330.80), $t(14)=4.52$, $P<0.001$.

3.4. Plasma prolactin responses to D-FEN and placebo

To determine the effect of fenfluramine administration compared to placebo on plasma prolactin concentration, data were again subjected to a 2 (group status)×12 (time) RM-ANOVA. RM-ANOVA revealed a significant main effect for drug (F (1, 13)=47.79, $P<0.001$), time (F (5, 65)=45.34, $P<0.001$), and drug×time interaction (F (5, 65)=17.18, $P<0.001$). Additionally, significant differences were found for area under the curve between prolactin response to D-FEN ($M=726.50$, S.D.=502.30) and placebo ($M=41.80$, S.D.=246.56), $t(14)=-5.88$, $P=0.00$.

3.5. Peak delta PRL [D-FEN] and peak delta OXY [D-FEN] responses

Timing of peak delta OXY and peak delta PRL responses to D-FEN were similar (mean for OXY [D-FEN]: 244±45 min; mean for PRL [D-FEN]=240±56 min). Peak prolactin and oxytocin concentrations in response to D-FEN challenge were z-transformed to account for their different units of measurement. No significant correlation was found between the z-transformed peak values ($r=0.204$, $n=14$, $P=0.49$).

3.6. Behavioral effects of D-FEN challenge

Subjective emotional and motivational state measured by Visual Analogue Scale included *talkative, happy, drowsy, nervous, sad, calm, depressed, anxious, fearful, mellow, irritable, high, angry, tired, hungry*, and *energetic*. To determine the effect of fenfluramine administration compared to placebo on emotional and motivational state, data were subjected to a 2 (group status)×6 (time) RM-ANOVA. After correcting for multiple comparisons, no significant drug, time, or drug×time interaction was detected. No significant drug or drug×time interaction was detected for temperature, although a significant effect of time was detected (F (5, 26)=14.438, $P<0.01$). Similarly, no significant drug or drug×time interaction was detected for heart rate, although a significant effect for time was detected (F (5, 26)=2.51, $P=0.038$). No significant drug, time, or drug×time interaction was detected for blood pressure.

3.7. Discussion

This study demonstrates that D-FEN, in comparison to placebo, stimulates the release of both oxytocin and prolactin into the plasma of healthy adult male subjects. The timing of these responses appears to be similar although there is no evidence of a relationship between the magnitudes of these two hormone responses. Since oxytocin is released directly from the hypothalamus, these data suggest that oxytocin response to 5-HT stimulation may be utilized as a truly central hormone outcome measure in pharmaco-challenge studies. If so, use of this measure will remove the pituitary as a 'window' into the brain in such studies.

The use of placebo control argues against the possibility that the results of this study are due to nonspecific environmental effects on hormone

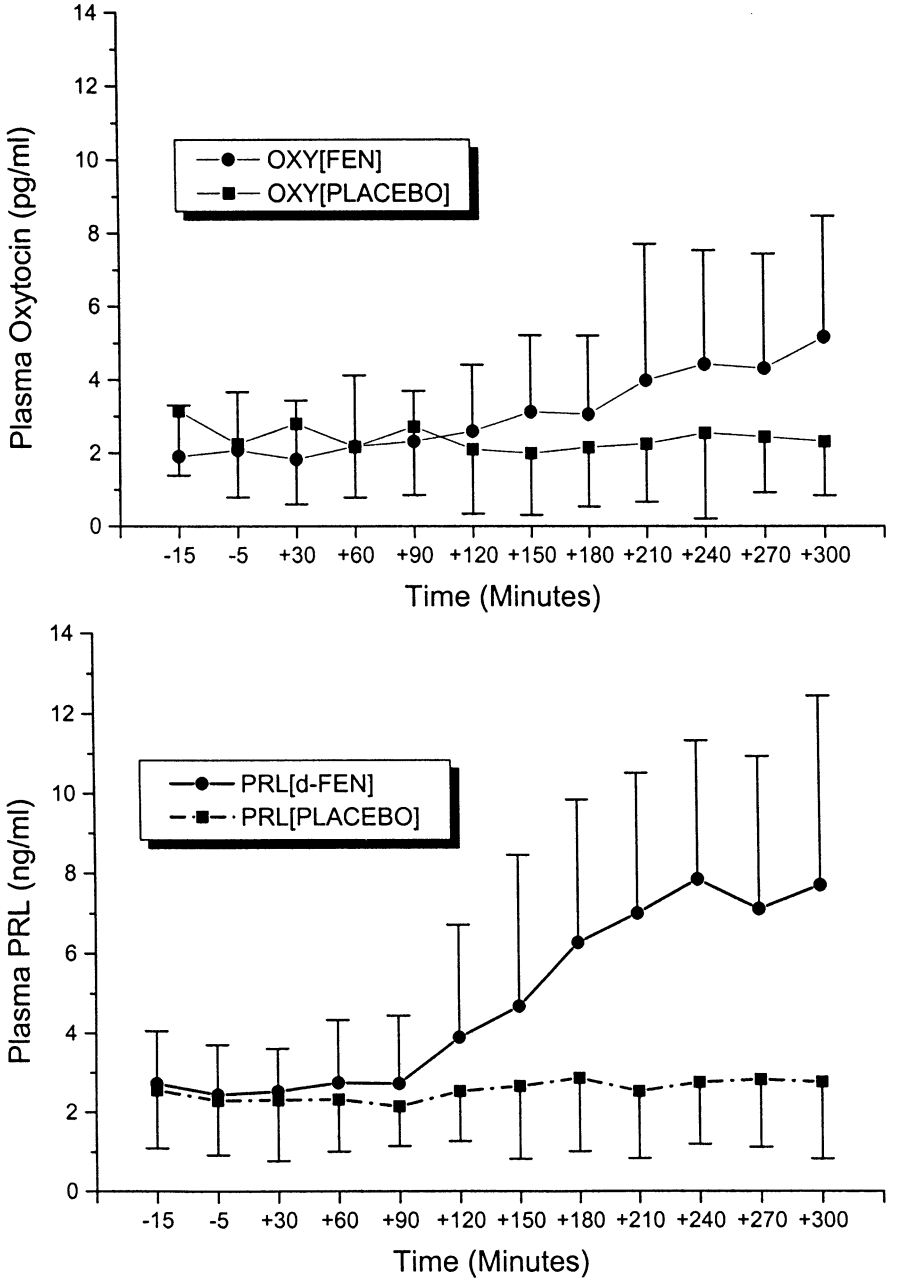

Fig. 1. OXY [D-FEN] (pg/ml) and OXY (PLACEBO) vs. time (min); PRL [D-FEN] (ng/ml) and PRL [PLACEBO] (ng/ml) vs. time (min).

secretion. In addition, the results of this study are not likely due to the known diurnal variation of plasma oxytocin levels. In adult males, oxytocin concentration peaks at 14:00 h. Basal oxytocin reaches a nadir in the late afternoon (Forsling et al., 1998), close to the time of peak oxytocin

response to D-FEN (14:00 h) observed in this study. Finally, no relationship was found for age and oxytocin response to D-FEN. This is in concordance with the findings of Chiodera et al. (1994), who reported no alteration in oxytocin secretion with age.

Three previous studies in human healthy volunteers have found mixed results using central 5-HT agents to stimulate oxytocin release. Low doses of the 5-HT-1_a agonist ipsapirone led to oxytocin response in males and females in one study (Cleare et al., 1998), but this positive result was not replicated in a placebo-controlled study (Newman et al., 1999). This may be because the oxytocin response to ipsapirone is dose dependent and the dose of ipsapirone necessary to release oxytocin is higher than that typically given to, or that can be tolerated by, human subjects (Li et al., 1994). A study in humans using the 5-HT_{2C} agonist *m*-CPP found no oxytocin response in males but a clear oxytocin response in females (Bagdy and Arato, 1998). One explanation for this apparent gender difference is that circulating estrogen plays a facilitatory role on hormonal response to 5-HT receptor stimulation in general (O'Keane et al., 1991) and on oxytocin release specifically (Griffen and Ojeda, 1988). It is possible that the oxytocin response to *m*-CPP is amplified in female subjects. Alternatively, it is possible that in males an *m*-CPP induced oxytocin response occurs centrally, but that this release of oxytocin cannot be detected in the plasma. For example, experimental stimuli such as social defeat, lead to an intrahypothalamic but not peripheral, release of oxytocin from the supra-optic nucleus (SON).

The effect of D-FEN on plasma oxytocin is most likely due to its effect on brain 5-HT. This is demonstrated by the fact that D-FEN's effect on central oxytocin release is blocked by acute pretreatment with the 5-HT transporter inhibitor fluoxetine (Javed et al., 1999). Since D-FEN enters 5-HT terminals through the 5-HT transporter, interference with the transporter stops uptake of D-FEN.

Some clues about which 5-HT receptor subtypes mediate D-FEN's effect on oxytocin release may be gleaned from studies of D-FEN on prolactin release. Based on receptor antagonist studies in animals, the prolactin response to D-FEN is most likely mediated by D-FEN stimulation of 5-HT_{2C} receptors (Di Renzo et al., 1989). This is also suggested by human studies utilizing relatively selective antagonists for 5-HT_{1A}, 5-$HT_{2A/C}$, and 5-HT_3 receptor subtypes (Park and Cowen, 1995; Coccaro et al., 1996a,b,c,d; Albinsson et al., 1994). Since the 5-HT_{2C} receptor agonist *m*-CPP causes a release of oxytocin (Bagdy, 1996), the oxytocin response to D-FEN demonstrated in this study may also be mediated by 5-HT_{2C} receptors. Further study would be necessary to rule out the role of other 5-HT receptor subtypes in the mechanism of D-FEN-induced OXY release.

Regarding the location of D-FEN's effect on oxytocin release, lesion studies in animals show that it is mediated by hypothalamic effects on both the parvocellular nucleus (PVN; Javed et al., 1999; Mikkelsen et al., 1999) and the SON (Javed et al., 1999). The PVN and SON rely on different 5-HT receptor subsets for oxytocin secretion. Animal studies utilizing selective 5-HT receptor antagonists suggest a role for both 5-HT_{1A} and 5-HT_{2A} receptors in the PVN (Van de Kar et al., 2001) and 5-HT_{2C} receptors in the SON (Bagdy, 1996; Bagdy and Makara, 1995). If oxytocin response to D-FEN is due primarily to 5-HT_{2C} receptor stimulation, it would follow that the oxytocin response to D-FEN seen in this study was due primarily to stimulation of the hypothalamic SON. However, since D-FEN is not a truly specific 5-HT_{2C} receptor agonist, and the role of other receptor subtypes cannot be ruled out, it would be premature to speculate that the oxytocin response in this study was a result of D-FEN's activity on the SON exclusive of the PVN.

There appeared to be no correlation between the oxytocin and prolactin response to D-FEN in the overall sample. This is not surprising, since the mechanisms of response of the two hormones to D-FEN stimulation are not identical. 5-HT_{2C} receptor mediated prolactin response is from the PVN (Bagdy and Makara, 1995), while 5-HT_{2C} receptor-mediated oxytocin response is from the SON (Bagdy and Makara, 1995). Other factors responsible for this difference include, but are not limited to, differences in the activation of specific 5-HT receptor subtypes that contribute to each hormonal

response, differences in the anatomical location of this activation, and differences in the nature of the specific hormonal systems.

In summary, we have found that D-FEN administration is associated with an oxytocin response in healthy human males and that this response parallels that of the known prolactin response to D-FEN in many ways, the two responses are not correlated. The positive results of this study, when compared to the more mixed results of studies using ipsapirone and *m*-CPP challenge, indicate that further work should be done to investigate the utility of oxytocin as a marker of central 5-HT function as well as the mechanism of oxytocin release in humans.

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