JPET Fast Forward. Published on March 2, 2005 as DOI: 10.1124/jpet.104.082073
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5-HT1A Receptors In The Paraventricular Nucleus of The Hypothalamus Mediate Oxytocin and Adrenocorticotropin Hormone Release as well as Some Behavioral Components of the Serotonin Syndrome

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Running Title Page

Running Title: 5-HT1A receptor-mediated endocrine responses in the PVN

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Number of text pages: 17

Number of tables: 0

Number of figures: 5

Number of references: 40

Number of words in

Abstract: 247

Introduction: 710

Discussion: 1,500

Abbreviations:

Mean arterial pressure (MAP)

Heart Rate (HR)

Paraventricular Nucleus of the Hypothalamus (PVN)

Adrenocorticotropin hormone (ACTH)

8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)

WAY100635 (WAY)

Recommended Section Assignment: Neuropharmacology

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Abstract

Neuroendocrine responses to administration of serotonin releasing agents or 5-HT1A-receptor agonists have been used as an index of serotonin receptor function in patients with depression and other mood disorders. However, the location and receptor population that mediates these responses have not been clearly identified. We tested the hypothesis that 5-HT1A receptors in the paraventricular nucleus of the hypothalamus (PVN) mediate the release of adrenocorticotropin hormone (ACTH) and oxytocin after administration of a selective 5-HT1A agonist in conscious rats. Low dose infusion (1 nmol/100 nl/side) of the selective 5-HT1A antagonist, WAY100635 (WAY), into the PVN blocked the rise in ACTH and oxytocin stimulated by low dose (30 nmol/kg), iv administration of the 5-HT1A agonist, 8-OH-DPAT (274 ± 53 vs. 70 ± 20 pg/ml, $p<0.01$ for ACTH and 10.7 ± 3.4 vs. 4.6 ± 0.7 pg/ml, $p<0.05$ for oxytocin after saline or WAY pre-treatment respectively). WAY did not influence the bradycardic effect of 8-OH-DPAT (-56 ± 7 vs. -54 ± 6 bpm after saline or WAY). 8-OH-DPAT treatment also elicited locomotor activation followed by hindlimb abduction and flat body posture. Surprisingly, WAY attenuated some aspects of locomotor activation and reduced the duration of hindlimb abduction elicited by the agonist (5.1 ± 0.9 vs. 0.3 ± 0.3 min for saline or WAY-treated rats). These data indicate that 5-HT1A-receptor stimulation in the PVN mediates the characteristic neuroendocrine response to serotonin agonist challenge. Moreover, they provide the first evidence that aspects of the behavioral serotonin syndrome are mediated by forebrain hypothalamic receptors.

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Introduction

Measurements of hormone release and thermoregulatory responses to acute challenge with serotonergic releasing agents or clinically-approved partial 5-HT1A receptor agonists have been used to assess the sensitivity of serotonergic function in patients suffering from various psychological disorders such as depression, obsessive compulsive disorder or post-traumatic stress (Lesch et al., 1990a; Lesch et al., 1991; Flory et al., 1998; Shapira et al., 2000; Rinne et al., 2000). Administration of serotonin releasing agents, as well as 5-HT1A-receptor agonists, produces a distinct profile of stress hormone release characterized, in part, by increased plasma levels of adrenocorticotropin hormone (ACTH), prolactin and cortisol in human subjects (Lewis and Sherman, 1984; Lewis and Sherman, 1985; Lesch et al., 1990a; Lesch et al., 1990b). Depressed patients demonstrate a blunted release of ACTH and cortisol in response to acute administration of partial 5-HT1A-receptor agonists (Lesch et al., 1990a; Shapira et al., 2000). Positron emission tomography studies demonstrate reduced 5-HT1A receptor binding in several brain regions in both depressed patients as well as those diagnosed with panic disorder (Neumeister et al., 2004; Sargent et al., 2000). These findings suggest that depression and possibly other mood disorders are associated with a reduction of 5-HT1A-receptor sensitivity and other down-stream signaling components of the 5-HT1A-receptor system. However, the population of 5-HT1A receptors that mediate the hormonal response is not clear. As such, the mechanisms by which a neuroendocrine challenge with serotonergic agonists elicits hormonal responses are not entirely clear. More selective 5-HT1A-receptor agonists (having higher affinity for the 5-HT1A receptor than the partial agonists used clinically) are often used in animal models to

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assess serotonergic function following long-term treatment with serotonin-modulating drugs such as antidepressants (D'Souza et al., 2002). From these studies, investigators have begun to characterize the underlying mechanisms of neuroendocrine responses to 5-HT1A-receptor agonist challenge. In the naive rat, systemic injection of the selective serotonin 5-HT1A-receptor agonist, 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), elicits dose-dependent increases in plasma levels of ACTH and oxytocin as well as robust increases in corticosterone, all of which are blocked by systemic administration of the selective 5-HT1A receptor antagonist, WAY100635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)-cyclohexane carboxamide) (Bagdy and Kalogeras, 1993; Bagdy, 1996; Vicentic et al., 1998; Critchley et al., 1994).

8-OH-DPAT is highly lipophilic and readily crosses the blood brain barrier and so is thought to mediate its endocrine effects by a direct action on receptors within the CNS (Hadrava et al., 1995). Unilateral injection of 8-OH-DPAT directly into the PVN of the rat elicits a significant elevation in ACTH that is blocked by systemic administration of the relatively non-selective, mixed β-adrenergic- and serotonin 5-HT1A- and 5-HT1B-receptor antagonist, pindolol (Bagdy and Makara, 1994; Pan and Gilbert, 1992). These data suggest that a post-synaptic 5-HT1A receptor population in the PVN mediates the neuroendocrine response to 5-HT1A-receptor agonist challenge.

More recent evidence has implicated pre-synaptic 5-HT1A receptors of the dorsal raphe in the endocrine response to 8-OH-DPAT. Global lesion of serotonergic neurons with p-chlorophenylalanine halved the release of ACTH in response to a relatively large dose of 8-OH-DPAT (100 μg/kg, iv). In the same study, injection of 8-OH-DPAT directly into the dorsal raphe caused a significant increase in ACTH that was similar in

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magnitude to the response observed after intra-PVN infusion of the drug (Bluet Pajot et al., 1995) . These data suggest that there may be separate sites that contribute to the endocrine response to 5-HT1A-receptor agonist injection.

Moreover, evidence suggests that 5-HT1A agonists may also have significant affinity for other receptor populations that could potentially mediate neuroendocrine responses to 5-HT1A agonist challenge (Castillo et al., 1995; Stowe and Barnes, 1998). Indeed, the majority of previous animal studies examining the receptor populations responsible for the neuroendocrine response to serotonin agonists have used relatively large doses of parenterally delivered drug that could have non-specific pharmacological actions. In contrast, the clinically approved oral serotonin agonists used in human neuroendocrine challenge studies have extremely low bioavailability. Thus, previous studies utilizing such large doses in animals may not effectively model the mechanisms that govern hormone responses seen in the human neuroendocrine challenge. Therefore, we set out to test the hypothesis that a relatively low dose of 8-OH-DPAT mediates neuroendocrine release of ACTH and oxytocin exclusively through activation of 5-HT1A receptors in the PVN. These data may have an important impact on the interpretation of neuroendocrine challenge tests and consequently, on the interpretation of studies investigating mechanisms responsible for the therapeutic effects of antidepressants.

Methods:

Animals

Male Sprague Dawley rats weighing between 300 and 350g (Harlan, Indianapolis, ID) were given *ad libitum* access to food and water for at least 1 wk prior to surgery. The

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housing facility was maintained at a constant temperature of 22±2°C with a light/dark cycle of 12:12 hrs. All experiments were conducted in accordance with the American Physiological Society Guiding Principles for Research Involving Animals and Human Beings (2002) and were approved by the University IACUC.

Surgery

Cannulations: At least 10 days before the experiment, rats were fitted with bilateral guide cannulae that terminated just dorsal to the PVN according to previously published methods (Zhang et al., 2004) . Briefly, following induction of anesthesia with ketamine/xylazine (100 mg/kg + 7 mg/kg respectively, i.p.), stereotaxic surgical procedures were employed to implant 25 ga. bilateral guide cannulae (Plastics One, Roanoke VA). The cannulae were placed according to the coordinates published by Paxinos and Watson, i.e., 0.6 mm lateral and 7.2 mm rostral to the interaural line and 6.1 mm ventral from the skull surface (Paxinos and Watson, 1997). All cannulae were cemented into place with dental acrylic and jewler's screws secured to the skull.

Vascular cathetersization: 2-4 days prior to the experiment, the rats were re-anesthetized (ketamine/xylazine, i.p. 100 mg/kg + 7 mg/kg respectively) again and implanted with bilateral femoral venous catheters as well as a unilateral femoral arterial catheter (PE-50 heat-welded to a length of PE-10) to enable systemic administration of drug, volume restitution following blood sampling and measurement of arterial pressure respectively.

Data Acquisition

During all experiments, arterial pressure and heart rate were recorded continuously on a Macintosh G4 Powerbook computer using PowerLab data acquisition

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software (Chart v. 4.2, ADinstruments, Colorado Springs, CO). The arterial pressure was measured with a disposable pressure transducer (Abbott Labs, North Chicago, IL) and a PowerLab bridge amplifier (ADinstruments, Grand Junction, CO). Heart rate was calculated on-line with the Chart software program using peak-to-peak detection of the pulse pressure wave. In a second study, behavior was recorded during the entire experiment using a digital video camera suspended directly above the cages. Video images were captured directly to a PC computer using Pinnacle Studio 8 software.

Experimental Design

All experiments were completed between 10:00 AM and 2:00 PM. In the first experiment, animals were randomized to one of 2 groups. Two animals, selected randomly from different groups were run simultaneously. The arterial catheters were connected to the remote recording equipment through an overhead swivel while the rats rested unrestrained in their home cage. Two venous lines were connected via the same swivel system to drug- or saline-filled syringes. A bilateral 33 ga. injector (Plastics one, Roanoke, VA), filled with saline or the 5-HT1A-receptor antagonist, WAY100635 (10 mM), was placed in each side of the guide cannula and secured in place with a screw cap. The injector extended 1.5 mm below the end of the guide cannula. The rats were then allowed to habituate to the instrumentation for at least 30 min.

At the start of the experiment, a 400 μl blood sample was withdrawn from the arterial catheter for determination of plasma ACTH. Five min later, simultaneous, bilateral 100 nl injections of WAY 100635 (10 mM), or saline, were delivered over one min with an infusion pump. Fifteen min later a second 400 μl blood sample was taken and the volume replaced with saline. Immediately after volume replacement, 8-OH-

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DPAT (30 nmol/kg) was administered through the iv line. Additional 400 µl blood samples were taken 5 and 15 min after 8-OH-DPAT administration. Blood samples were then centrifuged and the plasma removed and stored at -80ºC until radioimmunoassay for ACTH was performed.

The 30 nmol/kg dose of 8-OH-DPAT was chosen in order to provide a plasma concentration in the nanomolar range (as predicted by the distribution described by Hadrava et al., 1995) that would also elicit a significant hormone response. The concentration and volume of antagonist was chosen such that 1 nmol/side could be delivered in a sufficiently small volume to prevent diffusion into the ventricular system. We predicted that 1 nmol/side would effectively block 8-OH-DPAT binding in the brain if the 30 nmol/kg dose was distributed at the 5:1 ratio of brain to plasma reported in a previous study (Hadrava et al., 1995). To determine that antagonist was administered discretely and did not have effects elsewhere that could be attributed to leakage into the ventricular system, the bradycardic and hypotensive responses to 8-OH-DPAT were also assessed following intra-PVN injection of antagonist. The depressor and bradycardic effects of 8-OH-DPAT are due to activation of a hindbrain 5-HT1A receptor populations remote from the PVN and therefore should not be influenced by antagonist injection in the PVN, unless the antagonist diffuses into the ventricular system (Fozard et al., 1987).

In the second experiment, animals were randomly assigned to one of 4 groups treated either with 100 nl/side of saline or WAY100635 (10 mM), followed by or 8-OH-DPAT (30 nmol/kg, i.v.) or saline in a completely randomized design. Animals were treated as described in experiment one with some modifications. First, no blood samples were taken prior to PVN injection. Instead, a 1.4 ml blood sample was taken 10 min after

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PVN injection. The blood was immediately centrifuged and the plasma saved for later determination of ACTH and oxytocin. The red blood cells were resuspended in 700 μl of warmed saline (37º C) and re-infused 10 min after withdrawal. Five min later, 8-OH-DPAT (30 nmol/kg/200 μl, iv) or an equivalent volume of isotonic saline was administered. A second 1.4 ml blood sample was taken 5 min later. The re-suspended red blood cells were administered 10 min later and a final sample (1.4 ml) was taken 5 min after re-infusion of the red blood cells. The animals were sacrificed and their brains removed and placed in 4% paraformaldehyde overnight. The brains were subsequently submerged in 30% sucrose in PBS for at least 48 hrs after which they were sectioned (40 μm), mounted on slides and stained with cresyl violet for verification of proper cannula placement.

During the first experiment, we noted that behavioral responses characteristic of systemic 5-HT1A-receptor agonist administration were reduced in animals given PVN-injections of WAY100635. Therefore, during the second experiment, behavioral assessments were made in order to examine the possibility that 8-OH-DPAT acts in the hypothalamus to mediate the well known behavioral responses to 5-HT1A-receptor agonist administration such as locomotor activation and hindlimb abduction.

Data Analysis

Blood pressure and heart rate were averaged over 1 min intervals. Blood pressure and heart rate responses were assessed by comparing the maximal response to 8-OH-DPAT or saline observed 5 min following injection. Behavioral responses were assessed by determining the number of cage crossings, rears, full turns and grooming episodes (> 5 sec) that were observed during the 10 min period following iv 8-OH-DPAT- or saline

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injection. In addition, the length of time animals spent in a flat body posture was determined for 10 min immediately following injection. Two-WAY analyses of variance (ANOVA) were used to assess cardiovascular and behavioral responses. Endocrine responses were assessed by 2 and 3-WAY ANOVAs with repeated measures where appropriate. Post-hoc Newman- Keuls test were used to determine individual group differences.

Hormone Assays

Plasma ACTH and oxytocin concentrations were determined as described by Li et al. (Li et al., 1993).

Results

Three rats were excluded from the study due to placement of one of the cannula into the third ventricle or placement of either cannula more than 0.3 mm posterior or anterior to the margins of the PVN. Figures 1a and 1b demonstrate cannulae placement for all animals included in the first and second studies respectively.

Experiment 1

Hormone Responses

Baseline plasma ACTH levels, determined after habituation to the set-up but before drug intervention, were well within the range of low to normal values expected from unstressed animals (45 ± 12 pg/ml, Figure 2). Subsequent bilateral PVN injection of WAY100635 (1 nmol/100 nl/side) or saline did not alter ACTH levels. Intravenous injection of 8-OH-DPAT (30 nmol/kg) produced a highly variable, but significant rise in ACTH within 5 minutes of drug administration ($P<0.01$). ACTH levels were still

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elevated, but had declined by 15 min after the injection. Prior treatment with WAY100635 blocked the ACTH response to systemic injection of 8-OH-DPAT (Figure 2).

Cardiovascular Responses

8-OH-DPAT administration produced a pronounced bradycardic response that was maximal within 5 min of injection. The bradycardic response was accompanied by a mild hypotensive response. However, pre-treatment with WAY100635 had no effect on the cardiovascular responses to systemic 8-OH-PAT administration (-12.8 ± 2.5 vs. -12.8± 3.5 mmHg; -86 ± 12 vs. –75 ± 22 bpm for WAY100635 and saline pre-treated rats respectively).

Behavioral Responses

5-HT1A agonists are known to induce a characteristic set of behavioral responses (Green, 1984). However, it was not anticipated that such behavioral responses would be influenced by blockade of 5-HT1A receptors in the PVN. As a result, quantitative behavioral assessments were not made in the first experiments. Nonetheless, rats given 8-OH-DPAT were observed to have a very distinct set of behavioral responses that were noticeably absent from rats pre-treated with WAY100635. While the behavioral responses varied somewhat between animals, most demonstrated an initial period of locomotor activation that consisted of a transient period of orientation, followed by rearing and repeated cage crossings. In addition, the animals often made single rotations. This initial locomotor period typically lasted for 1-2 min and was eventually replaced by slowing of locomotion and initiation of hindlimb abduction that resulted in a flattened

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body posture. This posture was easily distinguished from the normal crouched posture exhibited by resting rats.

Experiment 2

Hormone Responses

In the second study, WAY100635 had no effect on either plasma ACTH or oxytocin levels 10 min after PVN injection (Figure 3). Subsequent systemic injection of 8-OH-DPAT significantly elevated ACTH levels ($P<0.01$), and had a mild but significant stimulatory effect on oxytocin release within 5 min of injection ($P<0.05$) that declined by 20 min after injection. Pre-treatment with WAY100635 in the PVN completely prevented the rise in ACTH ($P<0.01$) and oxytocin ($P<0.05$, Figure 3).

Cardiovascular Responses

As in experiment 1, both groups of rats treated with 8-OH-DPAT in experiment 2 developed significant bradycardia within 5 min ($P<0.01$) that was not affected by WAY100635 pre-treatment (Figure 4). Blood pressure rose slightly and transiently after 8-OH-DPAT injection (data not shown). The pressor response was coincident with locomotor activation, but was then reversed to a slight hypotensive response as the rats assumed a flat body posture. Even when the bradycardic response was maximal in 8-OH-DPAT-treated rats, the hypotensive response did not differ between animals given saline or 8-OH-DPAT (Figure 4). As in experiment 1, the blood pressure responses to 8-OH-DPAT did not differ between groups pre-treated with saline or WAY100635 in the PVN. Rats treated with systemic saline also showed some evidence of bradycardia compared to pre-injection baseline. However, this effect appeared to be related to recovery from the mild tachycardia that developed following reinfusion of re-suspended

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red blood cells performed just prior to pre-injection baseline measurements. The bradycardic response to 8-OH-DPAT was substantially greater (>50 bpm, $P<0.01$) than that observed following saline injection (Figure 4).

Behavioral Responses

Locomotor activation commonly developed within the first 30 seconds of iv 8-OH-DPAT administration and was characterized by active cage crossings and single rotations. Most 8-OH-DPAT-injected animals pre-treated with WAY100635 did not demonstrate locomotor activation. However, in some saline-pre-treated rats given 8-OH-DPAT, the initial locomotor period was truncated by the rapid onset of flat body posture. In such cases, fewer cage crosses were observed. In addition, one member of the saline control group demonstrated several cage crosses following the saline injection. Consequently, there was only a tendency for a significant effect of 8-OH-DPAT on cage crossings ($P=0.06$, Figure 5c). However, the numbers of single rotations was significantly increased after 8-OH-DPAT administration (Figure 5b). Within 1-2 minutes after 8-OH-DPAT administration any observable locomotor activation was typically replaced by flat body posture and hindlimb abduction. This posture usually lasted 5-10 minutes after which animals resumed a normal hunched posture typical of the untreated resting rat (Figure 5a). The effect of 8-OH-DPAT on both flat body posture ($P<0.01$) and turning behavior ($P<0.01$) was completely blocked by the pre-treatment with WAY in the PVN.

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Discussion

Systemically administered 8-OH-DPAT was found to stimulate 5-HT1A receptors in the PVN to mediate ACTH and oxytocin release. This is the first study to use low volume PVN infusion of the specific 5-HT1A-receptor agonist, WAY100635, to block systemically administered 5-HT1A-receptor agonist. Moreover, this is the first study to control for diffusion of antagonist into the cerebroventricular system by simultaneously evaluating responses mediated by 5-HT1A receptor populations in the more caudal hindbrain.

Relatively large doses of 5-HT1A receptor agonists have been used in past studies to implicate PVN 5-HT1A receptors in responses to neuroendocrine challenge. For instance, 10 μg of 8-OH-DPAT delivered in a 2 μl PVN infusion was found to raise ACTH levels in conscious rats (Pan and Gilbert, 1992). We noted that injections of fast green dye in volumes greater than 400 nl directed to the PVN invariably led to staining within the 4th ventricle (unpublished observation) indicating that the higher volume infusion may not provide discrete receptor stimulation. Nevertheless, cortisocsterone responses to a large (1 mg/kg) iv injection of the partial 5-HT1A receptor agonist, ipsapirone, was found to be attenuated in rats subjected to by bilateral lesion of the PVN (Bagdy and Makara, 1994). Welch et al. (1993) suggested that such large doses mediate the endocrine response by triggering reflex hormonal release following establishment of the cardiodepressor effect of 5-HT1A-receptor activation. In the present study, large increases in ACTH and mild increases in oxytocin were elicited with a relatively low dose of 8-OH-DPAT (~10 μg/kg) that had no significant effect on blood pressure. Thus, these neuroendocrine responses were likely not the result of baroreflex activation.

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Neuroendocrine responses might also be stimulated by non-serotonergic mechanisms. 8-OH-DPAT and other 5-HT1A agonists stimulate α1-adrenergic mediated vasoconstriction *in vivo* and *in vitro* (Castillo et al., 1993). Electrophysiological studies indicate that norepinephrine positively modulates glutamate induced EPSPs in magnocellular and parvocellular neurons of the PVN through activation of pre-synaptic α1-adrenergic receptors (Boudaba et al., 1996; Daftary et al., 2000). In previous work, a large (0.3 mg/kg), subcutaneous dose of 8-OH-DPAT failed to consistently raise ACTH levels following administration of the mixed dopamine, α1-adrenergic receptor antagonist, haloperidol (Gilbert et al., 1988). In the present study, we utilized a dose of 8-OH-DPAT previously found to have little effect on blood pressure following autonomic blockade (Osei-Owusu and Scrogin, 2004). As such, it is unlikely that neuroendocrine responses observed in the present study were related to activation of α1-adrenergic receptors in the PVN since the same dose of agonist had no apparent vasoconstrictor effect.

Some physiological responses elicited by 8-OH-DPAT are recognized to be mediated by 5-HT7 receptors (Hedlund et al., 2004). However, the endocrine responses to 8-OH-DPAT described here are not likely due to 5-HT7 receptor stimulation since the selective 5-HT1A-receptor antagonist, WAY100635, completely blocked the neuroendocrine response. Ligand binding studies indicate that WAY100635 effectively reveals high affinity binding sites with a 5-HT7 receptor-binding profile (Stowe and Barnes, 1998). Thus WAY100635 appears to have limited affinity for 5-HT7 receptors.

In the present study, low volume infusion of the selective 5-HT1A receptor antagonist, WAY100635, blocked the rise in both ACTH and oxytocin, but had no effect

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on the bradycardic response to systemic 8-OH-DPAT administration thought to be initiated by 5-HT1A receptors in the hindbrain (Fozard et al., 1987). As such, the present data indicate that the agonist likely acted within the vicinity of the PVN to mediate its effects on oxytocin and ACTH release since the antagonist apparently did not diffuse into the cerebroventricular system to influence 5-HT1A receptors in more caudal brain regions.

In our study, 8-OH-DPAT elicited only a minor elevation in oxytocin, but had a dramatic, though transient effect on ACTH levels. The discrepancy in the extent of hormone stimulation may reflect the amplification of the corticotropin releasing hormone response to 5-HT1A-receptor stimulation. In contrast, oxytocin is released in direct response to the 5-HT1A-receptor agonist and is not part of an amplification cascade. The dose of 8-OH-DAT used in the current study most likely produced peak brain levels that only just exceeded the threshold for hormone release. As a result the hormone responses were relatively short compared to the half-life (~20 min) of 8-OH-DPAT, presumably because brain levels of the drug declined below that sufficient to elicit further hormone release, possibly through some form of redistribution prior to its metabolism.

This report also provides the first evidence that 5-HT1A-receptor agonists act in the hypothalamus to mediate some of the classic behavioral responses attributed to 5-HT1A-receptor activation. The full behavioral serotonin syndrome is characterized by a complex set of behaviors that include hyperactivity, hindlimb abduction, head weaving, forepaw treading and straub tail. Investigators have used brain transection techniques to localize the hyperactivity and hindlimb abduction features of the behavioral syndrome to the activation of serotonin receptors in the brain stem or spinal cord (Deakin and Green,

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1978; Jacobs and Klemfuss, 1975). However, in these studies the behaviors were elicited by administration of tryptophan coupled with monoamine oxidase inhibition which increases extracellular serotonin levels. Previous brain transection studies must be interpreted with caution since such manipulations could sever motor outputs, as well as axons carrying newly synthesized serotonin to pre-motor sites that contribute to the motoric response. Interestingly, transection of the brain between the hypothalamus and the dorsal raphe was shown to abolish hindlimb abduction in response to tryptophan treatment (Jacobs and Klemfuss, 1975). The authors interpreted the findings to suggest that caudal, possibly serotonergic projections to motor outputs were severed, thereby disrupting the behaviors.

Alternatively, ascending afferent projections from the dorsal raphe to the hypothalamus might play a key role in initiating behavioral responses to serotonin by activating forebrain sites that provide descending projections to motor outputs of the brainstem and spinal cord. Locomotor activity is influenced by forebrain projections of the serotonergic system. Hillegaart et al. (1990) reported that injections of 5-HT1A-receptor agonist directly into the dorsal raphe inhibited spontaneous locomotor activity in rats. It was speculated that agonist activation of pre-synaptic 5-HT1A autoreceptors suppressed serotonergic neurons projecting to forebrain sites controlling motoric behavior (Hillegaart, 1990) . However, the serotonergic targets that mediate locomotor activation have so far not been identified.

In our studies, mild hyperactivity followed by flat body posture were elicited at a dose of 8-OH-DPAT that was relatively low compared to that used in most other animal neuroendocrine studies (Bagdy and Makara, 1994; Bagdy, 1996; Vicentic et al., 1998).

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Though none of these studies reported behavioral effects, the one previous report that explicitly examined the dose-dependent behavioral effects of 8-OH-DPAT showed responses only at much higher subcutaneous doses of drug (Hillegaart et al., 1996). It is possible that the apparent high potency of 8-OH-DPAT to produce behavioral effects when administered intravenously may result from a more rapid delivery of drug to the brain and thus possibly a higher peak 5-HT1A receptor occupancy than is achieved with similar doses of drug given subcutaneously. Since the dose of 8-OH-DPAT used in the current study elicited only very mild increases in oxytocin that appear to be just slightly above threshold, our data suggest that behavioral responses are elicited at similar, or possibly even lower doses than neuroendocrine responses. Whether this finding is dependent on the route of administration remains to be seen since, to our knowledge, no other studies have addressed both the behavioral and neuroendocrine responses simultaneously.

A much larger oral dose of the partial 5-HT1A-receptor agonist, ipsapirone (20 mg), given to healthy men produced a similar percent elevation in oxytocin as seen after the iv dose of 8-OH-DPAT used in the current study (Cleare et al., 1998). In contrast, oxytocin levels 10-fold higher are observed in neuroendocrine challenge tests in animals using the higher doses of 5-HT1A receptor agonist typical of theses studies (Vicentic et al., 1998). Investigators performing human studies have not reported evidence of any behavioral effects of ipsapirone. While, it is possible that observed behavioral effects were simply not reported in the human study, it is also possible that expression of behavioral responses to 5-HT1A-receptor activation may require much higher drug doses than hormone responses in humans. The partial 5-HT1A receptor agonists used in human

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neuroendocrine challenge tests have only about 5% bioavailability. Therefore, it is quite possible that the 5-HT1A-receptor occupancy achieved in our study with iv 8-OH-DPAT injection better reflects the 5-HT1A-receptor occupancy achieved in human neuroendocrine challenge tests. In contrast, most neuroendocrine studies in animals use drug doses that promote much higher, longer-lasting increases in oxytocin that may be due, in part, to activation of additional mechanisms that are not responsible for hormone release in human neuroendocrine challenge.

In conclusion, these data are consistent with the view that attenuated hormone responses to neuroendocrine challenge in depressed patients are due to reduced sensitivity of post-synaptic 5-HT1A-receptors in the PVN. Evidence of PVN 5-HT1A-receptor insensitivity may serve as a marker for more global alterations in 5-HT1A-receptor signaling that influence mood. Alternatively reduced sensitivity of PVN 5-HT1A receptors may directly impact mood by attenuating release of hormones with intrinsic anxiolytic and antidepressant properties such as oxytocin (Arletti and Bertolini, 1987; Windle et al., 1997).

Acknowledgement

The authors thank Francisca Garcia and Dr. Nicole Sullivan for technical assistance and Dr. Nancy Muma for helpful review of the manuscript.

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Foot Notes

This project is supported by grants from the American Heart Association, Mid-West Affiliate (0310026Z to POO) and NIH HLBI (HL076162 and HL072354 to KES).

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Portions of the data presented herein were published in abstract form in the proceedings of Experimental Biology 2003 meeting.

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Legends for Figures

Figure 1. Schematic representation of cannula placement for experiments 1 (A) and 2 (B). Schematic shows ventral half of representative coronal sections containing the PVN at rostral, caudal planes indicated in relation to Bregma. Symbols represent injections sites located at or between the section indicated and the next caudal section. Symbols are only indicated for one side of injection for clarity. ▲, Saline/Saline; ○, Saline/8-OH-DPAT; △, WAY100635/Saline; ●, WAY100635, 8-OH-DPAT.

Figure 2. Plasma ACTH before saline or WAY100635 (1 nmol/100nl/side) infusion in the PVN and immediately before-, 5 min- and 15 min after 8-OH-DPAT injection in conscious unrestrained rats. Data are groups means ± SEM, $^{**}p<0.01$ between groups.

Figure 3. Plasma ACTH (A) and oxytocin (B) before, 5 min and 20 min after injection with systemic 8-OH-DPAT (30 nmol/kg, iv) of saline (SAL) injection in conscious unrestrained rats pre-treated with saline (SAL) or WAY100635 (WAY, 1 nmol/100nl/side) infusion in the PVN. Data are group means ± SEM, $^{**}p<0.01$ SAL/DPAT vs. SAL/SAL, $^{*}p<0.05$ SAL/DPAT vs. all other groups.

Figure 4. Blood pressure and heart rate change 5 min after systemic injection of saline (SAL) or 8-OH-DPAT in rats pre-treated with SAL or WAY-100635 (WAY). Data are group means ± SEM, $^{**}p<0.01$ vs. SAL/SAL; $^{++}p<0.01$ vs. WAY/SAL

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Figure 5. Behavioral responses including numbers of cage crosses, minutes of flat body posture and individual turns during first 10 min after systemic saline (SAL) or 8-OH-DPAT injection (30 nmol/kg, iv) in animals pre-treated with SAL or WAY100635 (WAY) infusion in the PVN. Data are group means ± SEM, $^{**}p<0.01$ vs. all groups.

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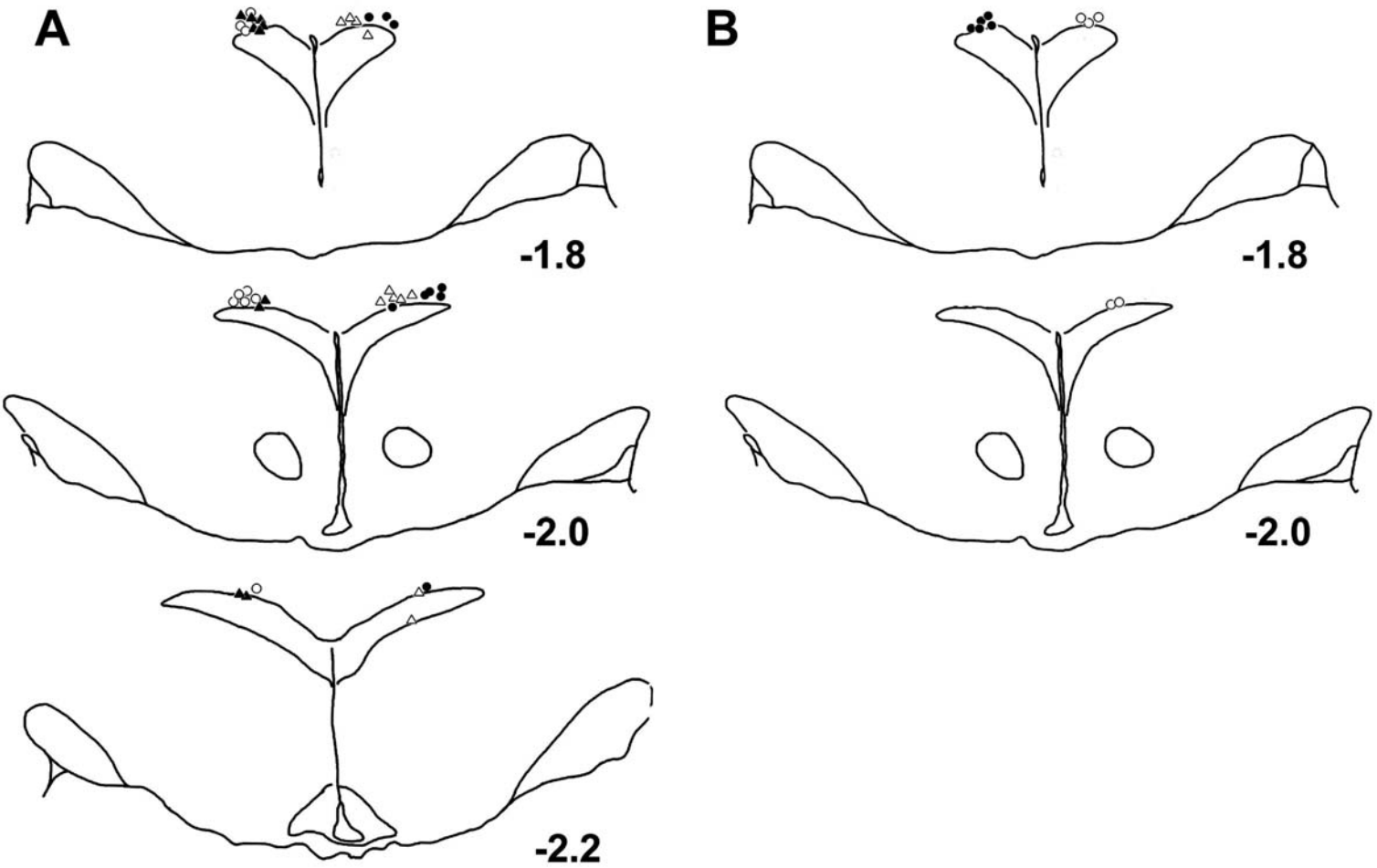

Figure 1

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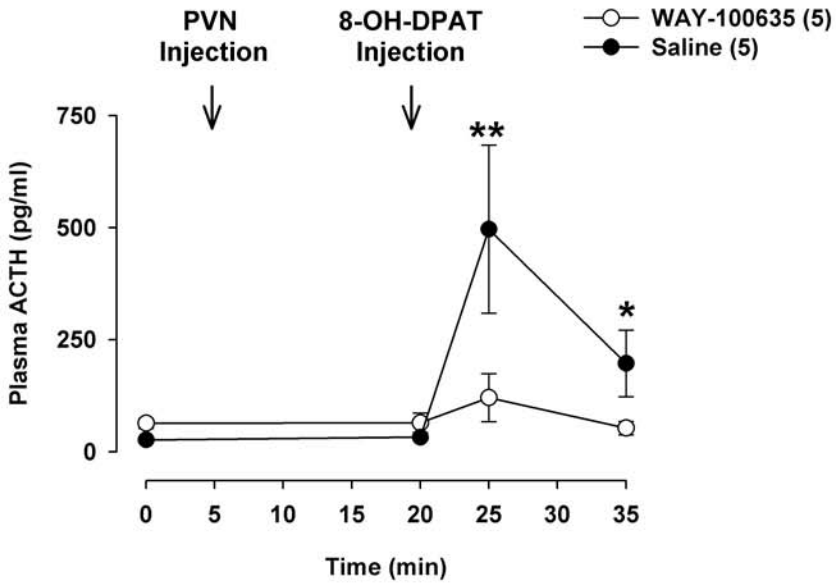

Figure 2

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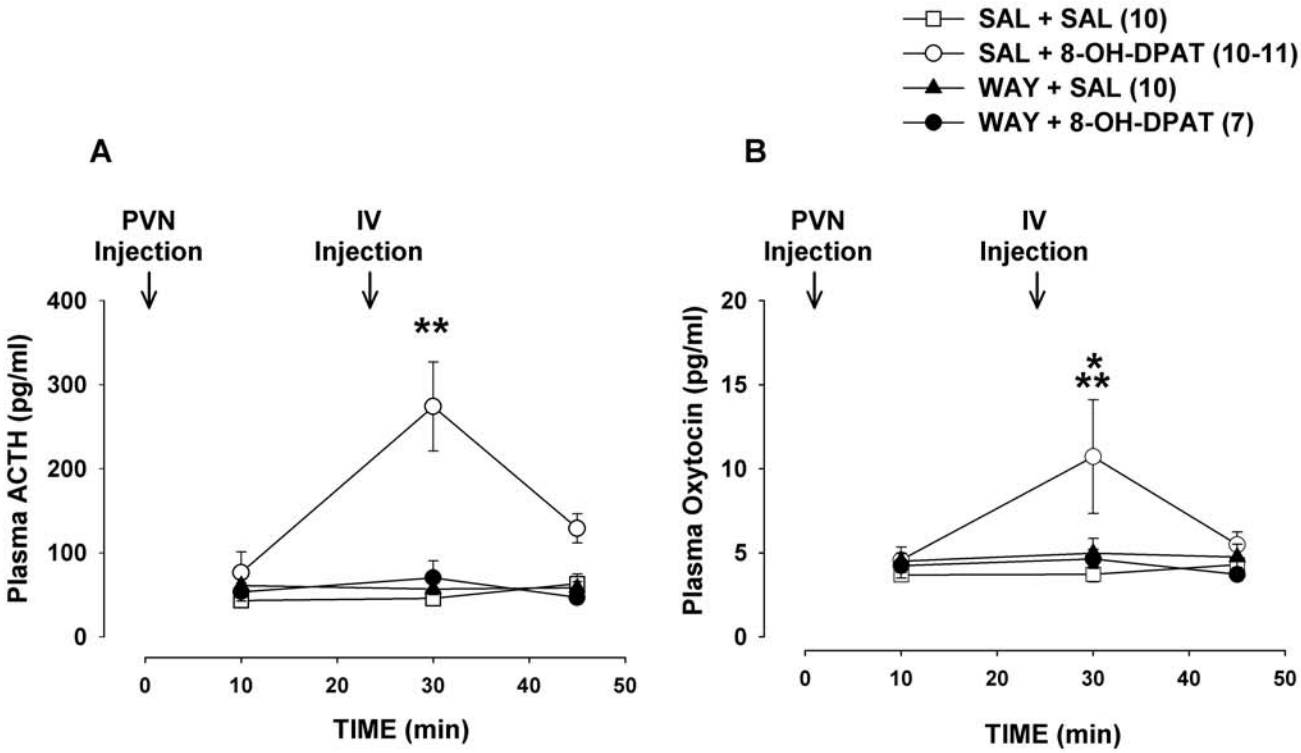

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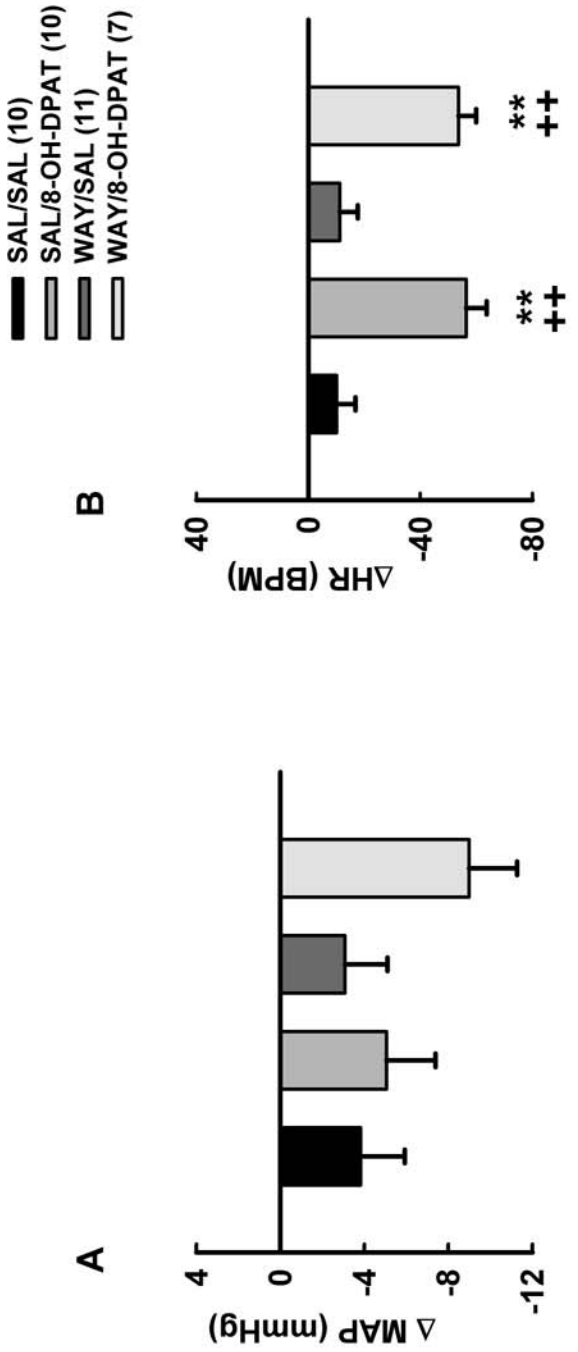

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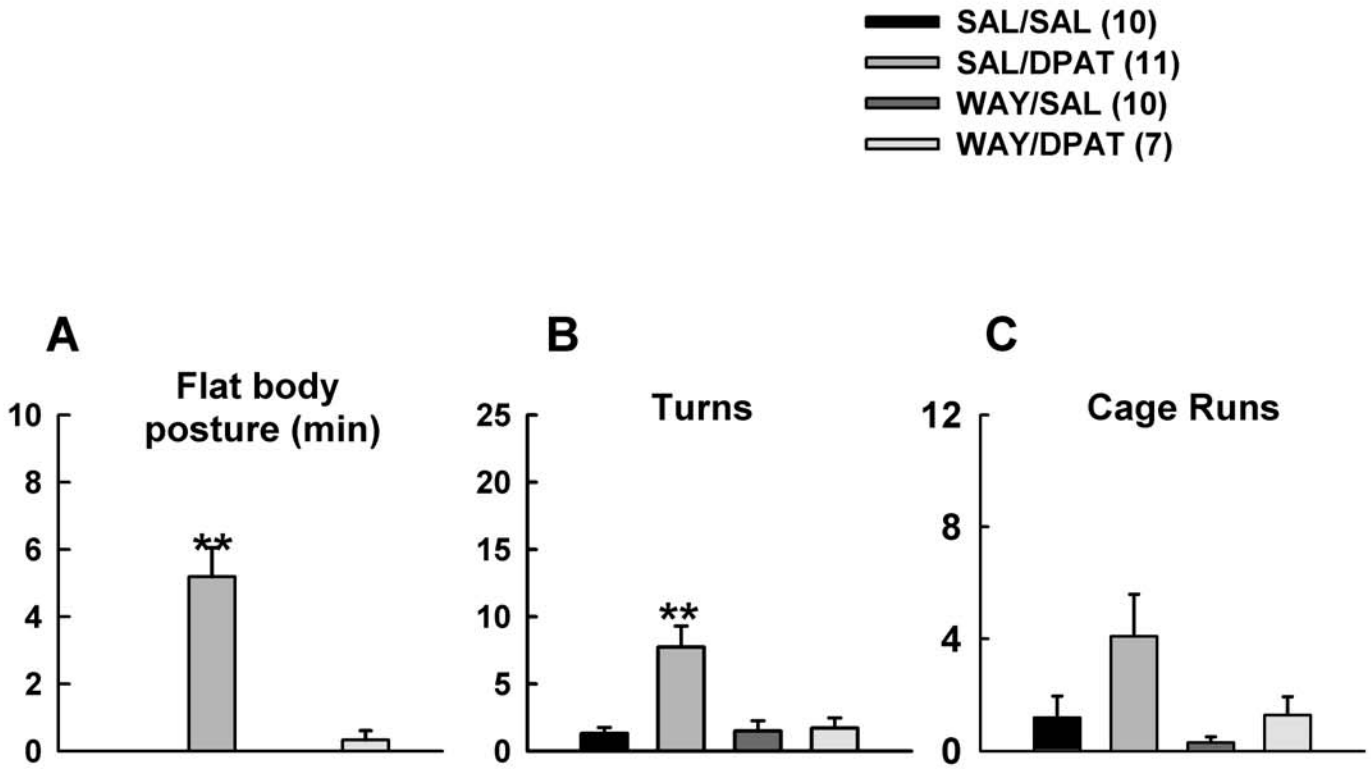

Figure 5

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