5-Hydroxytryptamine 1A Receptors in the Paraventricular Nucleus of the Hypothalamus Mediate Oxytocin and Adrenocorticotropic Hormone Release and Some Behavioral Components of the Serotonin Syndrome

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Received December 9, 2004; accepted February 23, 2005

ABSTRACT

Neuroendocrine responses to administration of serotonin releasing agents or 5-hydroxytryptamine (5-HT) 1A receptor agonists have been used as an index of serotonin receptor function in patients with depression and other mood disorders. However, the receptor population that mediates these responses has not been clearly identified. We tested the hypothesis that 5-HT1A receptors in the paraventricular nucleus of the hypothalamus (PVN) mediate the release of adrenocorticotropic 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, into the PVN blocked the rise in ACTH and oxytocin stimulated by low-dose (30 nmol/kg) i.v. administration of the 5-HT1A agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT); 274 ± 53 versus 70 ± 20 pg/ml, P < 0.01 for ACTH and 10.7 ± 3.4 versus 4.6 ± 0.7 pg/ml, P < 0.05 for oxytocin after saline or WAY pretreatment, respectively. WAY did not influence the bradycardic effect of 8-OH-DPAT (−56 ± 7 versus −54 ± 6 beats per minute after saline or WAY). 8-OH-DPAT treatment also elicited locomotor activation followed by hind limb abduction and flat body posture. Surprisingly, WAY attenuated some aspects of locomotor activation and reduced the duration of hind limb abduction elicited by the agonist (5.1 ± 0.9 versus 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.

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, 1991; Flory et al., 1998; Rinne et al., 2000; Shapira 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 adrenocorticotropic hormone (ACTH), prolactin, and cortisol in human subjects (Lewis and Sherman, 1984, 1985; Lesch et al., 1990a,b). 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 (Sargent et al., 2000; Neumeister et al., 2004). These findings suggest that depression and possibly other mood disorders are associated with a reduction of 5-HT1A receptor sensitivity and other downstream signaling components.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; ACTH, adrenocorticotropic hormone; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; WAY100635, [O-methyl-3H]-N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl)cyclohexanecarboxamidetrihydrochloride; PVN, paraventricular nucleus of the hypothalamus; bpm, beats per minute.

DOI: 10.1124/jpet.104.082073

This work was supported by American Heart Association, Mid-West Affiliate Grant 0100262Z (to P.O.-O.) and by National Heart, Lung, and Blood Institute Grants HL076162 and HL072354 (to K.E.S.). Portions of the data presented herein were published in abstract form in the proceedings of the Experimental Biology 2005 meeting.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.104.082073
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 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)tetratin (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 (Bagdy and Kalogeras, 1993; Critchley et al., 1994; Bagdy, 1996; Vicent et al., 1998).

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 central nervous system (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 nonselective, mixed β-adrenergic and serotonin 5-HT1A and 5-HT1B receptor antagonist, pindolol (Pan and Gilbert, 1992; Bagdy and Makara, 1994). These data suggest that a postsynaptic 5-HT1A receptor population in the PVN mediates the neuroendocrine response to 5-HT1A receptor agonist challenge.

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

Materials and Methods

Animals

Male Sprague-Dawley rats weighing between 300 and 350 g (Harlan, Indianapolis, IN) were given ad libitum access to food and water for at least 1 week prior to surgery. The housing facility was maintained at a constant temperature of 22 ± 2°C with a light/dark cycle of 12/12 h. 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 Institutional Animal Care and Use Committee.

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 + 7 mg/kg, respectively), stereotaxic surgical procedures were employed to implant 26-gauge bilateral guide cannulae (Plastics One, Roanoke, VA). The cannulae were placed according to the coordinates published by Paxinos and Watson (1997), i.e., 0.6 mm lateral and 7.2 mm rostral to the interaural line and 6.1 mm ventral from the skull surface. All cannulae were cemented into place with dental acrylic and jeweler’s screws secured to the skull.

Vascular Catheterization. Two to 4 days prior to the experiment, the rats were reanesthetized (ketamine/xylazine i.p., 100 + 7 mg/kg, respectively) 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 software (Chart version 4.2; ADinstruments, Colorado Springs, CO). The arterial pressure was measured with a disposable pressure transducer (Abbott Diagnostics, Abbott Park, IL) and a PowerLab bridge amplifier (ADinstruments). 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 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 two 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-gauge injector (Plastics One), filled with saline or the 5-HT1A receptor antagonist, WAY100635 (10 mM), was placed in 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 minutes later, simultaneous, bilateral 100-μl injections of
WAY100635 (10 mM), or saline, were delivered over 1 min with an infusion pump. Fifteen minutes later, a second 400-μl blood sample was taken, and the volume was replaced with saline. Immediately after volume replacement, 8-OH-DPAT (30 nmol/kg) was administered through the i.v. 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 was removed and stored at −80°C until radioimmunoassay for ACTH was performed.

The 30-nmol/kg dose of 8-OH-DPAT was chosen 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 hind brain 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 four groups treated either with 100 nl/side saline or WAY100635 (10 mM), followed by 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 PVN injection. The blood was immediately centrifuged, and the plasma was saved for later determination of ACTH and oxytocin. The red blood cells were resuspended in 700 μl of warmed saline (37°C) and reinforced 10 min after withdrawal. Five minutes later, 8-OH-DPAT (30 nmol/kg/200 μl i.v.) or an equivalent volume of isotonic saline was administered. A second 1.4-ml blood sample was taken 5 min later. The resuspended red blood cells were administered 10 min later, and a final sample (1.4 ml) was taken 5 min after reinfusion of the red blood cells. The animals were sacrificed, and their brains were removed and placed in 4% paraformaldehyde overnight. The brains were subsequently submerged in 30% sucrose in phosphate-buffered saline for at least 48 h, 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 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 hind limb 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 with 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 s) that were observed during the 10-min period following i.v. 8-OH-DPAT or saline 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 were used to assess cardiovascular and behavioral responses. Endocrine responses were assessed by two- and three-way analyses of variance 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. (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. Figure 1, A and B, demonstrate cannulae placement for all animals included in the first and second studies, respectively.

Experiment 1

Hormone Responses. Baseline plasma ACTH levels, determined after habitation 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, Fig. 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 min of drug administration (P < 0.01). ACTH levels were still 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 (Fig. 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, pretreatment with WAY100635 had no effect on the cardiovascular responses to systemic 8-OH-PAT administration (−12.8 ± 2.5 versus −12.8 ± 3.5 mm Hg; −86 ± 12 versus −75 ± 22 bpm for WAY100635- and saline-pretreated 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 was noticeably absent from rats pretreated with WAY100635. Although 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 to 2 min and was eventually replaced by slowing of locomotion and initiation of hind limb abduction that resulted in a flattened 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 (Fig. 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. Pretreatment with WAY100635 in the PVN completely prevented the rise in ACTH ($P < 0.01$) and oxytocin ($P < 0.05$, Fig. 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 pretreatment (Fig. 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 (Fig. 4).

Behavioral Responses. Locomotor activation commonly developed within the first 30 s of i.v. 8-OH-DPAT administration and was characterized by active cage crossings and single rotations. Most 8-OH-DPAT-injected animals pretreated with WAY100635 did not demonstrate locomotor activation. However, in some saline-pretreated 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 ($P = 0.06$, Fig. 5C). However, the numbers of single rotations was significantly elevated following systemic 8-OH-DPAT (30 nmol/kg i.v.) of saline (SAL) injection in conscious unrestrained rats pretreated with SAL or WAY100635 (WAY, 1 nmol/100 nl/side) infusion in the PVN. Data are group means ± S.E.M. $**$, $P < 0.01$ SAL/DPAT versus SAL/SAL, $*$, $P < 0.05$ SAL/DPAT versus all other groups.
significantly increased after 8-OH-DPAT administration (Fig. 5B). Within 1 to 2 min after 8-OH-DPAT administration, any observable locomotor activation was typically replaced by flat body posture and hind limb abduction. This posture usually lasted 5 to 10 min after which animals resumed a normal hunched posture typical of the untreated resting rat (Fig. 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 pretreatment with WAY in the PVN.

**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 antagonist, 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 $\mu$g of 8-OH-DPAT delivered in a 2-$\mu$l 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 fourth ventricle (K. E. Scrogin, unpublished data), indicating that the higher volume infusion may not provide discrete receptor stimulation. Nevertheless, corticosterone responses to a large (1 mg/kg) i.v. injection of the partial 5-HT1A receptor agonist, ipsapirone, was found to be attenuated in rats subjected to 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 $\mu$g/kg) that had no significant effect on blood pressure. Thus, these neuroendocrine responses were likely not the result of baroreflex activation.

Neuroendocrine responses might also be stimulated by nonserotonergic mechanisms. 8-OH-DPAT and other 5-HT1A agonists stimulate $\alpha_1$-adrenergic-mediated vasoconstriction in vivo and in vitro (Castillo et al., 1993). Electrophysiological studies indicate that norepinephrine positively modulates glutamate-induced excitatory postsynaptic potentials in magnocellular and parvocellular neurons of the PVN through activation of presynaptic $\alpha_1$-adrenergic receptors (Boudaba et al., 1996; Daftary et al., 2000). In previous work, a large (0.3 mg/kg) s.c. dose of 8-OH-DPAT failed to consistently raise ACTH levels following administration of the mixed dopamine, $\alpha_1$-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 $\alpha_1$-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 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, although 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 with 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, hind limb abduction, head weaving, forepaw treading, and Straub tail. Investigators have used brain transection techniques to localize the hyperactivity and hind limb abduction features of the behavioral syndrome to the activation of serotonin receptors in the brain stem or spinal cord (Jacobs and Klemfuss, 1975; Deakin and Green, 1978). 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 premotor sites that contribute to the motoric response. Interestingly, transection of the brain between the hypothalamus and the dorsal raphe was shown to abolish hind limb 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, ascendingafferent 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 (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 presynaptic 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 with that used in most other animal neuroendocrine studies (Bagdy and Makara, 1994; Bagdy, 1996; Vicentic et al., 1998). Although 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 s.c. 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 i.v. 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 s.c. 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 i.v. 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. Although 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 neuroendocrine challenge tests have only about 5% bioavailability. Therefore, it is quite possible that the 5-HT1A receptor occupancy achieved in our study with i.v. 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 postsynaptic 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).

Acknowledgments

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