Alterations in 8-Hydroxy-2-(dipropylamino)tetralin-Induced Neuroendocrine Responses after 5,7-Dihydroxytryptamine-Induced Denervation of Serotonergic Neurons

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ABSTRACT

In the present study, we examined denervation-induced changes in the sensitivity of hypothalamic postsynaptic serotonin_1A (5-HT_1A) receptor function with respect to changes in the dose-dependent elevation in plasma hormones [adrenocorticotropic hormone (ACTH), corticosterone, oxytocin, prolactin, renin and vasopressin] by the 5-HT_1A agonist 8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT). Rats received intracerebroventricular (i.c.v.) injections of the serotonin neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) or vehicle (0.1% ascorbate in saline) 3 weeks before challenge with increasing doses of 8-OH-DPAT (0, 10, 50 or 200 μg/kg s.c.). The effectiveness of 5,7-DHT-induced destruction of serotonergic neurons was confirmed by a 93% reduction in [3H]paroxetine-labeled 5-HT uptake sites in the hypothalamus. No changes in basal levels of ACTH, corticosterone, oxytocin, prolactin, renin and vasopressin were observed in rats that received i.c.v. 5,7-DHT injections. The dose-response curves for 8-OH-DPAT-induced elevations of plasma corticosterone and prolactin levels were shifted to the left in rats treated with 5,7-DHT, whereas no significant difference in the ACTH dose-response curve was observed between rats treated with vehicle and rats treated with 5,7-DHT. In contrast, the maximal oxytocin response to 8-OH-DPAT was attenuated in rats treated with 5,7-DHT. A 5,7-DHT-induced decline in the synthesis of oxytocin could explain this phenomenon. Although 8-OH-DPAT did not increase plasma levels of renin or vasopressin in rats treated with vehicle, 8-OH-DPAT produced an elevation (75%) in plasma renin concentration but not in vasopressin levels in rats that received i.c.v. injections of 5,7-DHT. No change was observed in [3H]8-OH-DPAT labeled 5-HT_1A receptors in the hypothalamus. In summary, denervation of hypothalamic serotonergic nerve terminals produces supersensitivity of some neuroendocrine responses to 8-OH-DPAT independent of changes in the density of hypothalamic 5-HT_1A receptors.

Serotonergic neurons innervate hypothalamic neurons that regulate the secretion of several hormones. Direct synaptic connections between serotonergic nerve terminals and CRH neurons in the hypothalamic PVN have been demonstrated at the electron microscopic level (Liposits et al., 1987). The CRH neurons in turn stimulate the secretion of corticotropin (ACTH) from the anterior lobe of the pituitary gland, which in turn stimulates corticosterone secretion from the adrenal cortex. Other evidence also points to direct serotonergic innervation of oxytocin-containing neurons in the hypothalamic PVN (Saphier, 1991; Kawano et al., 1992). Additionally, several lesion studies have provided evidence that the serotonergic stimulation of the secretion of ACTH, corticosterone, prolactin, oxytocin and renin is mediated by neurons in the hypothalamic PVN (Bagdy and Makara, 1994; Van de Kar et al., 1990, 1995; Bagdy, 1996; Rittenhouse et al., 1992b, 1993, 1994; Feldman et al., 1987; Gotoh et al., 1987). Destruction of the serotonergic nerve terminals that innervate the hypothalamic PVN can be expected to produce adaptive changes in postsynaptic 5-HT receptors.

Activation of 5-HT_1A receptors in the hypothalamic PVN stimulates the secretion of ACTH and oxytocin from the pituitary gland (Pan and Gilbert, 1992; Bagdy, 1996). Increased plasma levels of ACTH lead subsequently to the secretion of corticosterone from the adrenal gland. The magnitude of agonist-induced elevation in plasma ACTH and oxytocin levels can be used as a sensitive marker of the functional status of 5-HT receptor - signal transduction systems (Van de Kar, 1997). Indeed, we have previously observed that destruction of serotonergic nerve terminals in the

ABBREVIATIONS: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; 5-HT, 5-hydroxytryptamine (serotonin); CRH, corticotropin-releasing hormone; PVN, paraventricular nucleus; 8-OH-DPAT, 8-hydroxy-2-(dipropylamino)tetralin.
hypothesis that the magnitude of elevation in plasma levels of ACTH, corticosterone and oxytocin, after administration of the 5-HT1A agonist 8-OH-DPAT, as functional markers of postsynaptic 5-HT1A receptor systems.

Some 5-HT1A agonists, notably 8-OH-DPAT, also increase the secretion of prolactin (Bluet-Pajot et al., 1995; Meller and Bohmker, 1994; Kellar et al., 1992; Di Sciuolo et al., 1990; Poland, 1990; Aulakh et al., 1988; Carlsson and Eriksson, 1986). However, the prolactin response to 8-OH-DPAT and to other 5-HT1A agonists, such as ipsapirone, cannot be inhibited by 5-HT1A antagonists, suggesting that other receptors might mediate these effects (Aulakh et al., 1988; Seletti et al., 1995; Vicenzi et al., 1996). It is not clear whether these other receptors are 5-HT receptors or receptors for other neurotransmitters. If these are 5-HT receptor subtypes, desensitization of serotonergic nerve terminals would be expected to alter their sensitivity. For this reason, we measured the prolactin response to 8-OH-DPAT in rats whose serotonergic inputs into the hypothalamus were destroyed.

Administration of 5-HT1A agonists does not alter the secretion of renin or vasopressin in rats (Van de Kar et al., 1985; Lorens and Van de Kar, 1987; Rittenhouse et al., 1992a; Li et al., 1993, 1994; Brownfield et al., 1988; Bagdy et al., 1992). However, under some experimental conditions, for example, desensitization of 5-HT1A receptors by chronic treatment with 5-HT uptake inhibitors, 8-OH-DPAT or ipsapirone can increase plasma renin but not vasopressin levels (Li et al., 1993, 1994). The mechanism is not clearly understood; however, the effect of serotonergic denervation on the renin and vasopressin responses to 8-OH-DPAT was expected to shed some light on this phenomenon.

The destruction of serotonergic neurons by i.c.v. injections of 5,7-DHT was variably reported to increase or not to alter the density of 5-HT1A receptors in various forebrain regions, including the hypothalamus (Frankfurt et al., 1993, 1994; Pranzatelli, 1994; Miquel et al., 1992; Shimizu et al., 1992). In the present study, we also measured [3H]8-OH-DPAT-labeled 5-HT1A receptors in the hypothalamus. In addition, we verified the degree of denervation induced by 5,7-DHT by measuring the density of [3H]paroxetine-labeled 5-HT1A uptake sites. A reduction in [3H]paroxetine labeling would represent loss of serotonergic nerve terminals.

**Methods**

**Animals.** Male Sprague-Dawley rats (225–275 g) were purchased from Harlan (Indianapolis, IN). The rats were housed two per cage in a light- (12-hr light/dark cycle; lights on at 7:00 a.m) and temperature-controlled room. Food and water were available ad libitum. Eight to 10 rats were used per experimental group. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as approved by Loyola University Institutional Animal Care and Use Committee.

**Drugs.** All drug solutions were made immediately before injections. 5,7-DHT was obtained from Research Biochemical (Natick, MA) and dissolved in saline containing 0.1% ascorbic acid. 8-OH-DPAT was purchased from Research Biochemical, dissolved in saline and injected in doses of 10, 50 or 200 μg/kg s.c. in a volume of 1 ml/kg. Ampicillin and atropine methyl bromide were purchased from Sigma Chemical (St. Louis, MO). Noradrenalin was donated by Hoechst-Roussel Pharmaceuticals (Somerville, NJ).

**Intracerebroventricular injection of 5,7-DHT.** Serotonergic neurons were destroyed by stereotaxic i.c.v. injections of 5,7-DHT under pentobarbital anesthesia (50 mg/kg i.p.). All rats were pretreated with ampicillin (50 mg/kg i.m.) and atropine methylbromide (0.2 mg/kg i.p.). To prevent damage to dopaminergic or noradrenergic neurons, rats also received 15 mg/kg i.p. of the dopamine and noradrenaline uptake inhibitor nomifensin 20 min before i.c.v. injections of 5,7-DHT. Hamilton 25-μl syringes were lowered into the lateral cerebral ventricles (A, −0.5; L, 1.4; H, −4.6 from bregma), and 75 μg/10 μl/side of 5,7-DHT or vehicle were infused bilaterally over 1 min. The rats lost body weight in the first few days after surgery. We fed them sweet food (apples, sweetened water in dishes) to help them recover. By the third week after surgery, their body weights were similar to those of the vehicle-treated rats. There was no difference in body weight between saline- and 8-OH-DPAT-challenged rats.

**Experimental procedure.** The surgeries were staggered toward the day on which the animals were killed. All the rats were injected with saline or 8-OH-DPAT and killed 15 min later on the same day, between 10:30 and 13:10 a.m. (one rat every 2 min). After recovery from surgery (3 weeks), rats received injections of 8-OH-DPAT (0, 10, 50 or 200 μg/kg s.c.) and were decapitated 15 min later. Trunk blood was collected in centrifuge tubes containing 0.5 ml of a 0.3 M EDTA (pH 7.4) solution. Plasma was stored at −70°C until assayed for plasma hormone levels. To confirm the loss of 5-HT1A nerve terminals induced by 5,7-DHT, the brains were rapidly removed, and the hypothalamus was dissected and frozen at −70°C. The extent of the lesion was determined by measuring [3H]paroxetine binding to the 5-HT1A uptake sites in rat hypothalami (Battaglia et al., 1987).

**Radioimmunoassay for plasma hormone concentrations.** Plasma ACTH, corticosterone, prolactin and renin were measured by radioimmunoassays as described in detail in our previous report (Li et al., 1993). Plasma oxytocin and vasopressin were determined using radioimmunoassays detailed in our previous reports (Brownfield et al., 1988; Saydoff et al., 1991).

**Radioligand binding assay for 5-HT uptake sites in the hypothalamus.** Hypothalamic homogenates were prepared according to the protocol of Leonhardt et al. (1992). Briefly, frozen tissues were placed in a minimum of 25 volumes of ice-cold 50 mM Tris-HCl (pH 7.7, 25°C) containing 0.5 mM EDTA and 10 mM MgSO4 and homogenized using a Tekmar Tissumizer (2× 5 sec). The homogenate was centrifuged at 37,000 × g for 15 min at 4°C. Pellet was resuspended in 30 volumes of buffer, homogenized, incubated at 37°C for 15 min and then centrifuged at 37,000 × g for 10 min. Tissue was then washed and centrifuged once more. Finally, the pellet was resuspended to a volume of 30 mg wet wt/ml in cold 50 mM Tris-HCl (pH 7.7) containing 0.5 mM EDTA and 10 mM MgSO4. The protein concentration of the homogenate was measured according to Lowry et al. (1951). Paroxetine is a 5-HT uptake blocker that binds with a high affinity to 5-HT transporters (Battaglia et al., 1991; Dechant and Clissold, 1991; Dewar et al., 1991). Because 5-HT transporters are expressed by serotonergic neurons, a reduction of 5-HT transporters can be interpreted as loss of serotonergic nerve terminals (Battaglia, 1990). [3H]Paroxetine (20.3 Ci/mmol, DuPont NEN, Boston, MA) was incubated for 120 min at room temperature with hypothalamic homogenates (1 mg) at a final concentration of 0.44 nM in 5-ml total volume of a buffer containing 50 mM Tris HCl, 120 mM NaCl, and 5 mM KCl (pH = 7.7) according to a previously described protocol (Battaglia et al., 1987). Nonspecific binding was defined in the presence of 10 μM citalopram. The reaction was stopped by immediate filtration over Whatman GF/C filters and washed three times with 5 ml of a 50 mM Tris buffer (pH 7.7). The radioactivity of
the filters was counted in 5 ml of scintillation liquid at 56% efficiency.

Radioiodin binding assays for 5-HT<sub>1A</sub> receptors in the hypothalamus. Hypothalamic (1.5 mg wet tissue/tube) homogenates were incubated with 1 ml of Tris buffer (50 mM, pH 7.7, with 10 mM MgSO<sub>4</sub>, 0.5 mM EDTA, 10 µM pargyline and 0.02% ascorbate acid) containing [3H]8-OH-DPAT (129.5 Ci/mmol, 1 mCi/ml, DuPont NEN) in a concentration of 1.49 nM (K<sub>D</sub> concentration) at room temperature for 1 hr. Nonspecific binding was defined in the presence of 10 µM 5-HT. The reaction was stopped by immediate filtration over Whatman GF/C filters and washed three times with 5 ml of a 50 mM Tris buffer (pH 7.7). The radioactivity of the filters was counted in 5 ml of scintillation liquid at 56% efficiency.

Statistics. The data are presented as group mean and S.E.M. values. The data from the hormone assays were analyzed by a two-way ANOVA. Group mean values were compared with the use of the Newman-Keuls multiple-range test. The data obtained from radioligand binding analysis of [3H]paroxetine and [3H]8-OH-DPAT (comparing only two groups: lesion vs. vehicle) were analyzed with a two-sided t test (Steel and Torrie, 1960). A computer program (NWA STATPAK, Portland, OR) was used for all the statistical analyses.

Results

Intracerebroventricular injection of 5,7-DHT produced a marked (93%) reduction in the density of [3H]paroxetine-labeled 5-HT<sup>uptake</sup> sites in the hypothalamus, confirming the effectiveness of this lesion (table 1). In addition, evaluation of basal plasma hormone levels revealed no differences between rats treated with 5,7-DHT and vehicle-injected controls (see figures described below).

Injection of 8-OH-DPAT to the vehicle-pretreated rats produced a dose-dependent increase in plasma levels of ACTH (fig. 1A). In 5,7-DHT-treated rats, 8-OH-DPAT produced a similar dose-response curve. Although a small potentiation of the increase in levels of ACTH was observed at the 50 µg/kg dose of 8-OH-DPAT in 5,7-DHT-treated rats (317.4 ± 62.8) compared with vehicle-treated rats (242.2 ± 54.8), this was not statistically significant.

In rats treated with 5,7-DHT, there was a shift to the left of the dose-response effect of 8-OH-DPAT on plasma corticosterone concentration with no change in the maximal response (fig. 1B). The potentiation of the corticosterone response was statistically significant at the 50 µg/kg dose of 8-OH-DPAT, corresponding to the small but statistically non-significant potentiation of the ACTH response to the same dose of 8-OH-DPAT.

The prolactin dose-response to 8-OH-DPAT also was significantly shifted to the left in rats treated with 5,7-DHT compared with vehicle-injected controls (fig. 2). In addition, the maximal prolactin response to 8-OH-DPAT was higher in rats treated with 5,7-DHT. This contrasts with the corticosterone response, which showed no increase in the maximal response to 8-OH-DPAT. There was no relationship among the hormone responses to 8-OH-DPAT. For example, rats that had the highest prolactin response to 8-OH-DPAT had a corticosterone response in the middle of the group. Other rats had low prolactin response, whereas the corticosterone response was among the highest in the group.

The effects of 8-OH-DPAT on the neurohypophysial hormones oxytocin and vasopressin are shown in figure 3. Although 8-OH-DPAT produced a dose-dependent increase in

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<th>Treatment</th>
<th>n</th>
<th>[3H]Paroxetine binding pmol/gm tissue</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>38</td>
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</tr>
<tr>
<td>5,7-DHT</td>
<td>39</td>
<td>2.11 ± 0.66</td>
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Data represent the mean ± S.E.M.; n represents the number of rats per group. *(Significant difference from the vehicle group, P < .01 (Student’s t test).*
plasma oxytocin levels in vehicle-treated rats, treatment with 5,7-DHT significantly reduced the maximal oxytocin response to 8-OH-DPAT (fig. 3A). In contrast to oxytocin, 8-OH-DPAT did not alter plasma vasopressin levels in any groups (fig. 3B).

Injections of 8-OH-DPAT to vehicle-pretreated rats did not increase plasma renin activity (fig. 4A) or renin concentration (fig. 4B). However, in 5,7-DHT-treated rats, the highest dose of 8-OH-DPAT significantly increased both plasma renin activity and concentration (fig. 4).

Evaluation of [3H]8-OH-DPAT-labeled 5-HT1A receptors in hypothalamic homogenates revealed no differences between rats treated with 5,7-DHT and their controls (table 2).

**Discussion**

The results of the present study suggest that denervation of serotonergic nerve terminals in the hypothalamus results in supersensitivity of receptors mediating the effects of 8-OH-DPAT on several hormones, including corticosterone, prolactin and renin. The two exceptions are the neurohypophysial hormones vasopressin, which does not respond at all to 8-OH-DPAT, and oxytocin, which showed a reduced maximal response to 8-OH-DPAT in rats with 5,7-DHT-induced lesions in serotonergic neurons. Furthermore, none of these changes in hormone responses to a 5-HT1A agonist were accompanied by changes in [3H]8-OH-DPAT-labeled 5-HT1A receptors in the hypothalamus.

5,7-DHT is a relatively selective neurotoxin that can destroy serotonergic neurons in rats without destroying noradrenergic or dopaminergic neurons if these neurons are protected by pretreatment with the norepinephrine and dopamine uptake blocker nomifensin (Pileblad and Carlsson, 1985; Manias and Taylor, 1983). The dose of 5,7-DHT used in the present study has previously produced 85% depletion of hypothalamic 5-HT but did not decrease the norepinephrine and dopamine content of the hypothalamus (Van de Kar et al., 1981). [3H]Paroxetine labels serotonergic nerve terminals. Changes in this parameter parallel changes in the concentrations of 5-HT in the forebrain (Battaglia et al., 1987). The dose of 5,7-DHT used in the present study has previously produced 85% depletion of hypothalamic 5-HT but did not decrease the norepinephrine and dopamine content of the hypothalamus (Van de Kar et al., 1981). [3H]Paroxetine labels serotonergic nerve terminals. Changes in this parameter parallel changes in the concentrations of 5-HT in the forebrain (Battaglia et al., 1987). The dose of 5,7-DHT used in the present study has previously produced 85% depletion of hypothalamic 5-HT but did not decrease the norepinephrine and dopamine content of the hypothalamus (Van de Kar et al., 1981).

5-HT1A receptors play a major role in the serotonergic regulation of ACTH, corticosterone and oxytocin secretion (Bagdy, 1996; Fletcher et al., 1996; Critchley et al., 1994; Meller and Bohmaker, 1994; Pan and Gilbert, 1992; Li et al., 1993; Bagdy and Kalogeras, 1993; Bagdy and Makara, 1994). The presence of 5-HT1A receptors in the PVN has been demonstrated autoradiographically (Li et al., 1997a, 1997b), sug-

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**Fig. 3.** Alterations by i.c.v. 5,7-DHT of the dose-response effect of 8-OH-DPAT (0, 10, 50 and 200 μg/kg s.c.) on (A) plasma oxytocin and (B) plasma vasopressin levels. The data represent the mean ± S.E.M. of 8–10 rats per group. The results of the ANOVA for oxytocin are main effect of 5,7-DHT, $F_{1,68} = 10.76$ (P < .01); main effect of 8-OH-DPAT, $F_{3,68} = 29.36$ (P < .01); interaction between 5,7-DHT and 8-OH-DPAT, $F_{3,68} = 6.08$ (P < .01). The results of the ANOVA for vasopressin are main effect of 5,7-DHT, $F_{1,67} = 12.76$ (P < .01); main effect of 8-OH-DPAT, $F_{3,67} = 3.78$ (P < .05); interaction between 5,7-DHT and 8-OH-DPAT, $F_{3,67} = .34$ (NS). * Significant effect of 8-OH-DPAT (P < .05, Newman-Keuls test). #, Significant effect of 5,7-DHT (P < .05, Newman-Keuls test).

**Fig. 4.** Alterations by i.c.v. 5,7-DHT of the dose-response effect of 8-OH-DPAT (0, 10, 50 and 200 μg/kg s.c.) on (A) plasma renin activity and (B) plasma renin concentration. The data represent the mean ± S.E.M. of 8–10 rats per group. The results of the ANOVA are main effect of 5,7-DHT, $F_{1,68} = 12.76$ (P < .01); main effect of 8-OH-DPAT, $F_{3,68} = 3.78$ (P < .05); interaction between 5,7-DHT and 8-OH-DPAT, $F_{3,68} = .34$ (NS). #, Significant effect of 5,7-DHT (P < .05, Newman-Keuls test).

**TABLE 2**

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<th>Treatment</th>
<th>n</th>
<th>[3H]8-OH-DPAT binding pmol/gm tissue</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>2.93 ± 0.26</td>
</tr>
<tr>
<td>5,7-DHT</td>
<td>10</td>
<td>2.47 ± 0.18</td>
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Data represent the mean ± S.E.M.; n represents the number of rats per group.
gesting that these receptors directly stimulate the secretion of CRH and oxytocin. Because 5-HT₁A receptors are linked through G proteins to second messenger enzymes, each receptor can stimulate the release of multiple molecules of oxytocin and CRH. CRH also is linked via G proteins to effector enzymes. Hence, activation of each CRH receptor on corticotrophs in the pituitary will lead to the release of multiple molecules of ACTH, which can further stimulate the release of even more molecules of corticosterone (or cortisol in humans). This amplification of responses means that these hormones can be used as highly sensitive peripheral markers of the overall functional status of 5-HT₁A receptor systems in the hypothalamus. The leftward shift in the dose-response effect of 8-OH-DPAT on plasma corticosterone is consistent with the classic theory of denervation supersensitivity. Although no significant changes were observed in the 8-OH-DPAT dose-response curve for plasma ACTH, the small potentiation observed at the 50 μg/kg dose of 8-OH-DPAT could be sufficient to trigger a strong potentiated response of corticosterone release from the adrenal cortex. Similar exaggerated responses of corticosterone in response to small changes in plasma ACTH levels, particularly at plasma levels below 300 pg/ml have been reported by other investigators as well (Kaneko et al., 1980, 1981; Engeland et al., 1981; Bagdy et al., 1989).

Of great interest is the contrast between the potentiated hypothalamic-pituitary-adrenal axis and the reduced oxytocin response to 8-OH-DPAT. This reduced oxytocin response to 8-OH-DPAT was primarily a reduction in the maximal responsiveness, which could be due to reduced efficacy of 5-HT₁A agonists at oxytocin neurons or an overall reduction in responsiveness of the oxytocin neurons. Previous studies indicate that destruction of serotonergic neurons with i.c.v. 5,7-DHT reduces the levels of oxytocin in the pituitary gland and reduces the oxytocin responsiveness to other stimuli, such as osmotic stimulation (Saydoff et al., 1993). Similarly, depletion of brain 5-HT with PCPA reduces the oxytocin response to another stimulus, milk ejection reflex in lactating rats, and reduces the mRNA encoding oxytocin in the hypothalamus (Moos and Richard, 1983; Carter and Murphy, 1989). Combined, these studies suggest that oxytocin-containing neurons are sustained by serotonergic innervation, but other interpretations also are possible. This latter phenomenon suggests that oxytocin may be a uniquely sensitive peripheral marker of changes in hypothalamic serotonergic neurotransmission. This would make oxytocin a good peripheral marker for neuroendocrine challenge tests in neuropsychiatric disorders associated with serotonergic dysfunction.

The interpretation of the prolactin data is not as easy as it would seem at first glance. Although injection 8-OH-DPAT increases plasma prolactin levels, this effect cannot be inhibited by 5-HT₁A antagonists, suggesting that other receptors for which 8-OH-DPAT has a high affinity could mediate this effect (Aulakh et al., 1988; Seletti et al., 1995; Vicent et al., 1996). 8-OH-DPAT has a high affinity for hypothalamic 5-HT₇ receptors (Kᵢ = 47 nM) (Sleight et al., 1995) and 5-HT₅ receptors (Kᵢ = 46 nM) in cell culture (Wisdem et al., 1993). Therefore, it is possible that the prolactin response to 8-OH-DPAT may be mediated by activation of either of these receptors or of as-yet-unidentified receptors. The fact that supersensitivity occurs after serotonergic denervation suggests that a subclass of 5-HT receptors mediates the effect of 8-OH-DPAT on the secretion of prolactin. 8-OH-DPAT is a weak dopamine agonist, an effect that could inhibit the prolactin response to activation of 5-HT receptors. Although dopamine D₂ receptors in the pituitary exert a primary control on the secretion of prolactin, there is no evidence that destruction of serotonergic neurons will affect their sensitivity.

The administration of 8-OH-DPAT to rats normally does not result in increased renin release (Lorens and Van de Kar, 1987; Bagdy et al., 1992). However, several conditions have been reported to result in exposing a stimulatory effect of 8-OH-DPAT on the secretion of renin. Such conditions include desensitization of 5-HT₁A receptors by chronic treatment with fluoxetine (Li et al., 1993) and blockade of 5-HT₁A receptors with WAY-100635 (Vicent et al., 1996). However, supersensitivity of 5-HT₁A receptors, observed in rat offspring who received in utero cocaine injections, also exposes a stimulation by 8-OH-DPAT of renin release (Bataggia and Cabrera, 1994). The present results suggest that denervation of serotonergic neurons, which leads to supersensitivity of 5-HT₁A receptor-mediated ACTH response, also leads to stimulation of renin release by 8-OH-DPAT. Considering the fact that both supersensitivity and desensitization of 5-HT₁A receptors produce an increase in the renin response to 8-OH-DPAT, it is possible that 5-HT₁A receptors are involved in both inhibitory and stimulatory mechanisms that regulate renin release. Another possibility is that other 5-HT receptor subtypes for which 8-OH-DPAT has a high affinity might be involved. However, it is presently unclear which receptors could mediate this effect.

Both vasopressin and oxytocin are produced in cells in the hypothalamus and are released from their nerve terminals in the neural lobe of the pituitary gland. Serotonergic neurons can stimulate the secretion of both oxytocin and vasopressin (Bagdy et al., 1992; Iovino and Steardo, 1985; Brownfield et al., 1988; Pergola et al., 1993; Saydoff et al., 1993, 1996). Nevertheless, there are differences between these two hormones. The serotonergic regulation of vasopressin secretion does not seem to involve 5-HT₁A receptors, because all the 5-HT₁A agonists tested so far have failed to increase plasma vasopressin levels (Li et al., 1993, 1994; Brownfield et al., 1988; Bagdy et al., 1992). The present data indicate that even when serotonergic nerve terminals in the hypothalamus are destroyed, 8-OH-DPAT failed to stimulate the secretion of vasopressin, which makes this hormone unique among the hormones that respond to serotonergic stimulation.

The inability of long-term destruction of serotonergic neurons to alter the binding of [³H]8-OH-DPAT to hypothalamic 5-HT₁A receptors is surprising, particularly because previous autoradiographic analyses reported an increase the density of 5-HT₁A receptors in specific hypothalamic nuclei, such as the ventromedial nucleus (Frankfurt et al., 1993). On the other hand, several studies agree with the present results, that no change in 5-HT₁A receptor number can be observed after destruction of serotonergic neurons (Pranzatelli, 1994; Miquel et al., 1992; Hensler et al., 1991). One possible explanation is that perhaps only 5-HT₁A receptors in specific target neurons in subregions of the hypothalamus are up-regulated and that these changes would be difficult to observe in whole hypothalamic homogenates.

In conclusion, the present data suggest that denervation of serotonergic nerve terminals in the hypothalamus results in supersensitivity of 5-HT₁A receptors that activate the hypo-
thalamic-pituitary-adrenal gland. In addition, other 5-HT receptors that may regulate the secretion of prolactin and renin also become supersensitive. The oxytocin response to 8-OH-DPAT is decreased, possibly because of reduced oxytocin stores in the perikarya and in their nerve terminals in the neural lobe of the pituitary gland.

References


