

5-HT_{2A} Receptors Stimulate ACTH, Corticosterone, Oxytocin, Renin, and Prolactin Release and Activate Hypothalamic CRF and Oxytocin-Expressing Cells

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The 5-HT_{2A/2C} agonist (\pm)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) stimulates hypothalamic neurons to increase the secretion of several hormones. This study addressed two questions: 1) are the neuroendocrine effects of DOI mediated via activation of 5-HT_{2A} receptors; and 2) which neurons are activated by 5-HT_{2A} receptors. The 5-HT_{2A} antagonist (+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol (MDL 100,907; 0.001, 0.01, or 0.1 mg/kg, s.c.) was administered before rats were challenged with DOI (2.5 mg/kg, i.p.). MDL 100,907 produced a dose-dependent inhibition (ED₅₀ \cong 0.001 mg/kg) of the effect of DOI on plasma levels of ACTH, corticosterone, oxytocin, prolactin, and renin without altering basal hormone levels. Complete blockade of the effect of DOI was achieved for all hormones at MDL 100,907 doses of 0.01–0.1 mg/kg. In a parallel experiment, DOI was injected 2 hr before killing to determine its effects on the expression of Fos, the product of the immediate early gene

c-fos. DOI induced an increase in Fos immunoreactivity in corticotropin-releasing factor (CRF) and in oxytocin-expressing neurons but not in vasopressin-containing neurons in the hypothalamic paraventricular nucleus or CRF cells in the amygdala. Pretreatment with MDL 100,907 (0.1 mg/kg, s.c.) blocked the DOI-induced increase in Fos expression in all regions including the hypothalamus, amygdala (central and corticomedial), bed nucleus of the stria terminalis, and prefrontal cortical regions. The combined neuroanatomical and pharmacological observations suggest that the neuroendocrine responses to DOI are mediated by activation of neurons in the hypothalamic paraventricular nucleus and associated circuitry. Furthermore, selective activation of 5-HT_{2A} receptors mediates the hormonal and Fos-inducing effects of DOI.

Key words: serotonin; ACTH; oxytocin; MDL 100,907; *c-fos*; Fos; prolactin; renin; corticosterone

Dysfunction of serotonergic neurons is associated with neuropsychiatric disorders such as depression, anxiety, and premenstrual syndrome (Joffe and Cohen, 1998; Lucki, 1998). Alterations in serotonin₂ (5-HT₂) receptors may underlie some of the above disorders (Hollander et al., 1991; Massou et al., 1997; Sargent et al., 1998).

The neuroendocrine responses to serotonergic activation have been used as a diagnostic tool to examine the functioning of serotonergic neurons in the brains of patients suffering from mood disorders (Cowen, 1998). The serotonergic neurons that innervate neuroendocrine control regions in the hypothalamic paraventricular nucleus send collaterals to other limbic brain regions, notably the amygdala (Petrov et al., 1994). Thus, altered hormone responsiveness to specific 5-HT agonists could also reflect changes in other limbic brain regions.

5-HT₂ receptors are subdivided into 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors (Kroeze and Roth, 1998). (\pm)-1-(2,5-Dime-

thoxy-4-iodophenyl)-2-aminopropane HCl (DOI), a 5-HT_{2A/2C} agonist, has been used in rats to examine the role of 5-HT₂ receptors in the secretion of several hormones. Until recently, there were few pharmacological means to distinguish between the effects of DOI on 5-HT_{2A} and 5-HT_{2C} receptors (Rittenhouse et al., 1991, 1993; Saydoff et al., 1991; Bagdy et al., 1992). For example, spiperone has a higher affinity for 5-HT_{2A} than for 5-HT_{2C} receptors, but it is not useful to determine prolactin responses because it maximally increases prolactin secretion by antagonizing dopamine D₂ receptors on lactotrophs in the pituitary (Levy et al., 1995; Kurashima et al., 1996). The selective 5-HT_{2A} antagonist (+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol (MDL 100,907) (Dudley et al., 1990; Sorensen et al., 1993) is useful to evaluate the relative contributions of 5-HT_{2A} receptors to all neuroendocrine effects of DOI.

Fos, the protein product of the immediate early gene *c-fos*, is considered a reliable marker of synaptic activation (Hoffman et al., 1993; Luckman, 1995). Magnocellular neurons that produce oxytocin (and vasopressin) and corticotropin-releasing factor (CRF) neurons express Fos in response to a wide range of excitatory stimuli (hyperosmolarity, hypovolemia, hypotension, stress, D-fenfluramine, and dopamine agonists) (Verbalis et al., 1991; Olson et al., 1992; Hoffman et al., 1993; Eaton et al., 1996; Javed et al., 1999). Hence, *c-fos* expression in oxytocin and CRF-secreting neurons closely parallels CRF and oxytocin secretion, and Fos can be considered a sensitive marker of magnocellular secretory activity in response to several stimuli.

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The location of neurons that are activated by DOI has been mapped using Fos as a marker for neuronal activity (Leslie et al., 1993b; Rouillard et al., 1996). However, similar to the uncertainty regarding the role of 5-HT_{2A} and 5-HT_{2C} receptors in the neuroendocrine responses to DOI, there is a lack of insight into the identity of the 5-HT₂ receptor subtypes mediating the DOI-induced increase in Fos expression (Leslie et al., 1993b; Rouillard et al., 1996).

The present study used a combination of DOI and MDL 100,907 to clarify the role of 5-HT_{2A} receptors in neuroendocrine function and map the location of neurons that are activated by 5-HT_{2A} receptors. We hypothesize that: 1) 5-HT_{2A} receptors mediate the release of ACTH, corticosterone, oxytocin, prolactin, and renin; and 2) 5-HT_{2A} receptors induce a distinct pattern of Fos immunoreactivity in neurons of the hypothalamus that reflects their function in the release of these hormones.

MATERIALS AND METHODS

Animals. Adult male Sprague Dawley rats (225–275 gm) were purchased from Harlan Laboratories (Indianapolis, IN). Animals were housed two per cage in a light- (12 hr light/dark cycle, lights on at 7 A.M.), humidity-, and temperature-controlled room for at least 7 d before the experiment. Food and water were available *ad libitum*. Each experimental group consisted of eight animals for the neuroendocrine experiment and four rats per group for the Fos experiment. All procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* as approved by the Loyola University Institutional Animal Care and Use Committee.

Drugs. DOI was purchased from Research Biochemicals (Natick, MA) and was injected (2.5 mg/kg, i.p.). DOI was dissolved in saline and injected in a volume of 1 ml/kg. MDL 100,907 was a gift from Hoechst Marion Roussel Research Institute (Cincinnati, OH) and was injected subcutaneously at doses of 0.001, 0.01, and 0.1 mg/kg. MDL 100,907 was dissolved in a minimal volume of 0.01N HCl, diluted with saline, and injected in a volume of 1 ml/kg.

Experiment 1: neuroendocrine challenge tests. MDL 100,907 was administered subcutaneously at doses of 0.001, 0.01, and 0.1 mg/kg 1 hr before killing. The doses of MDL 100,907 were carefully selected to avoid occupancy of 5-HT_{2C} receptors. For example, a subcutaneous dose of 0.63 mg/kg was ineffective in blocking the discriminative stimulus properties of the 5-HT_{2C} agonist Ro 60–0175 (Dekeyne et al., 1999; Smith et al., 1999). Furthermore, MDL 100,907 produced a similar dose-dependent inhibition of the corticosterone response to two other 5-HT₂ agonists, quipazine and MK-212 (Hemrick-Luecke and Fuller, 1996). Rats were subsequently administered either saline or DOI (2.5 mg/kg, i.p.) 15 min before killing. This dose of DOI was shown previously to produce close to a maximal neuroendocrine response (Li et al., 1992, 1993a; Rittenhouse et al., 1993, 1994). The time interval for killing after DOI injection was based on data (Bagdy, 1996) indicating that plasma levels of ACTH, oxytocin, and prolactin reach a peak at 15–30 min. The rats were killed by decapitation, and trunk blood was collected in centrifuge tubes containing 0.5 ml of a 0.3 mM EDTA solution, pH 7.4. Plasma aliquots were stored at –70°C until hormone levels were determined via radioimmunoassays. Plasma oxytocin, ACTH, corticosterone, prolactin, and renin concentrations were examined by radioimmunoassays previously described in detail (Li et al., 1993b, 1997).

Experiment 2: Fos immunoreactivity. In the immunocytochemical experiment, the rats first received an injection of MDL 100,907 (0.1 mg/kg, s.c.) and 45 min later, received an injection of DOI (2.5 mg/kg, i.p.). Two hours after the second injection, the rats were overdosed with sodium pentobarbital (45 mg/kg, i.p.). Then the rats were perfused through the ascending aorta with 50 ml of 0.01 M PBS, pH 7.6, at room temperature, followed by 150 ml of the fixative 4% paraformaldehyde in 0.1 M phosphate buffer, pH 8.5, at 4°C. The brains were removed and placed in the fixative overnight at 4°C. Then the brains were cryoprotected by storage in 0.1 M phosphate buffer containing 30% sucrose for 2 d. The brains were frozen on dry ice and cut into 30 μm coronal sections on a cryostat. Sections were collected in 0.01 M PBS, and three alternating sections were stored at 4°C pending immunocytochemical analysis.

Immunocytochemistry. Fos immunocytochemistry was performed first. The sections were incubated with a polyclonal Fos antibody raised in

rabbits (Santa Cruz Biotechnology, Santa Cruz, CA) that was diluted 1:10,000 in 0.1 M Tris-buffered saline containing 0.5% Tween 20 (Fisher Scientific, Hanover Park, IL) and 1% normal goat serum for 2 d at room temperature. Then sections were rinsed and incubated in a solution containing 2% normal goat serum and goat anti-rabbit immunoglobulin G conjugated to biotin (Kirkegaard & Perry Laboratories, Gaithersburg, MD) for 1.5 hr at room temperature. Sections were washed and placed in streptavidin–peroxidase conjugate (Kirkegaard & Perry Laboratories) for 1 hr at room temperature. Fos immunoreactivity was detected using nickel ammonium sulfate intensified 3,3'-diaminobenzidine (Sigma, St. Louis, MO) as the chromogen. Fos immunoreactivity was localized to the cell nuclei and appeared as a dark gray–black stain.

CRF-expressing neurons were detected using a rabbit polyclonal anti-CRF antiserum (provided by J. A. Olchowska, University of Rochester, Rochester, NY). Oxytocin- and vasopressin-expressing neurons were detected using rabbit polyclonal anti-oxytocin and anti-vasopressin serum or anti-peptide E serum (marker for proenkephalin; provided by S. J. Watson, University of Michigan). Sections already stained for Fos immunoreactivity were washed first in 0.01 M PBS, pH 7.6, containing 0.25% Triton X-100 (Fisher Scientific). The sections were placed in 0.01 M PBS containing 0.3% hydrogen peroxide for 30 min. Sections were incubated for 2 d at room temperature with antisera in 0.01 M PBS containing 0.25% Triton X-100 and 1% normal goat serum at the following dilutions: anti-CRF, 1:5000; anti-oxytocin, 1:8000; all other antisera, 1:2000. Immunoreactivity for these peptides was visualized using the same biotinylated second antibody, streptavidin–peroxidase conjugate, and 3,3'-diaminobenzidine chromogen as described above, except that nickel ammonium sulfate was not present. Then sections were washed in 0.01 M PBS, mounted on gelatin-coated slides, and coverslipped with Permount mounting medium (Fisher Scientific). CRF, oxytocin, and vasopressin immunoreactivity was localized to the cell cytoplasm and was visible as a brown stain.

Immunocytochemical analysis. Sections obtained from all of the animals were matched for comparable rostrocaudal level. Brain regions were identified via a light microscope with the aid of the Paxinos and Watson atlas (1986). Fos label was identified by the characteristic light gray- to black-stained nuclear label seen in Fos positive cell bodies. Fos staining was counted bilaterally in the paraventricular and supraoptic nuclei of the hypothalamus; one section from the rostral, middle, and caudal segments of the paraventricular and supraoptic nuclei was sampled from each animal. The cell counts obtained from the three sections were combined as a total for the paraventricular or supraoptic nucleus of each subject. Neurons are considered to be double-labeled (i.e., both Fos-immunoreactive and peptide-immunoreactive) if they exhibit a light gray to black stained nucleus surrounded by a brown cytoplasm.

Statistical analysis. Hormone and Fos data were analyzed by two-way ANOVA, followed by the Newman–Keuls multiple range test (Steel and Torrie, 1960), using a computer statistical program (GB-STAT; Dynamic Microsystems, Inc., Silver Spring, MD). All data are represented as group means with the SEM.

RESULTS

Experiment 1: neuroendocrine study

As noted in Figures 1–3, DOI injection increased plasma levels of ACTH (1350%), corticosterone (355%), oxytocin (548%), prolactin (1456%), plasma renin activity (537%), and plasma renin concentration (415%). Administration of MDL 100,907 (0.001, 0.01, or 0.1 mg/kg, s.c.) did not alter the basal levels of these hormones (in saline-challenged rats). However, MDL 100,907 produced a dose-dependent inhibition of the effect of DOI on the levels of ACTH (Fig. 1A), corticosterone (Fig. 1B), oxytocin (Fig. 2A), prolactin (Fig. 2B), plasma renin activity (Fig. 3A), and plasma renin concentration (Fig. 3B). The oxytocin, prolactin, and renin responses to DOI were completely blocked by the 0.01 mg/kg dose of MDL 100,907 (Figs. 2, 3). All hormone responses to DOI were completely blocked by the subcutaneous 0.1 mg/kg dose of MDL 100,907. The ED₅₀ value for MDL 100,907 for inhibiting the release of ACTH, oxytocin, prolactin, and renin was ~0.001 mg/kg injected subcutaneously.

The two-way ANOVA for ACTH indicated a main effect of MDL 100,907 ($F_{(3,53)} = 28.47$; $p < 0.01$), a main effect of DOI

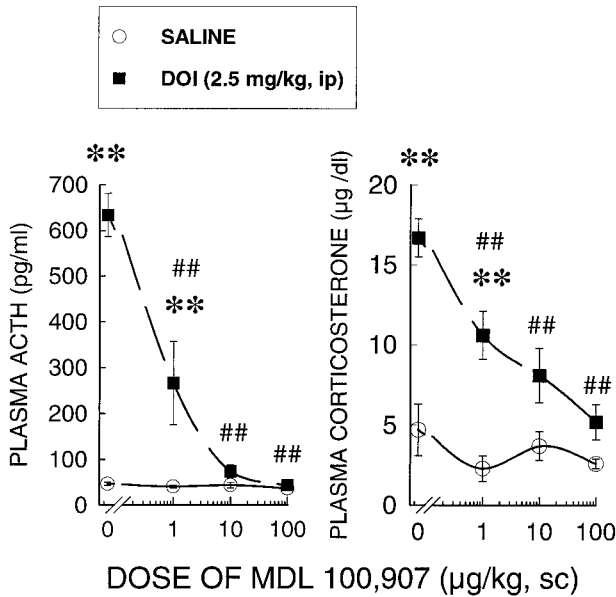


Figure 1. The effect of increasing doses of MDL 100,907 on plasma ACTH (left) and corticosterone (right) levels in rats challenged with either saline or DOI (2.5 mg/kg, i.p.). Circles represent rats challenged with saline, whereas squares represent rats challenged with DOI (2.5 mg/kg, i.p.). The data represent the mean \pm SEM of eight rats per group. **Significant effect of DOI, $p < 0.01$; ## significant effect of MDL 100,907, $p < 0.01$ (2-way ANOVA and Newman–Keuls multiple range test).

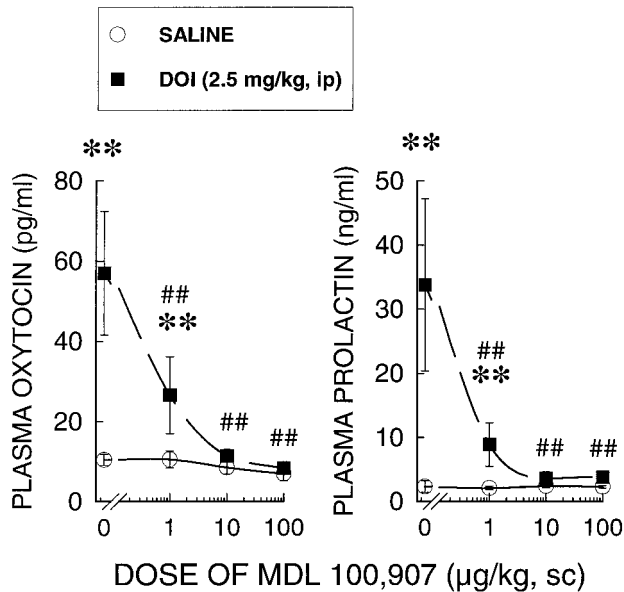


Figure 2. The effect of increasing doses of MDL 100,907 on plasma oxytocin (left) and prolactin (right) levels in rats challenged with either saline or DOI (2.5 mg/kg, i.p.). Circles represent rats challenged with saline, whereas squares represent rats challenged with DOI (2.5 mg/kg, i.p.). The data represent the mean \pm SEM of eight rats per group. **Significant effect of DOI, $p < 0.01$; ## significant effect of MDL 100,907, $p < 0.01$ (2-way ANOVA and Newman–Keuls multiple range test).

($F_{(1,53)} = 68.14$; $p < 0.01$), and a significant interaction between MDL 100,907 and DOI ($F_{(3,53)} = 27.14$; $p < 0.01$). For corticosterone, the main effect of MDL 100,907 ($F_{(2,54)} = 10.08$; $p < 0.01$), main effect of DOI ($F_{(1,54)} = 62.81$; $p < 0.01$), and interaction between MDL 100,907 and DOI ($F_{(3,54)} = 5.76$; $p < 0.01$)

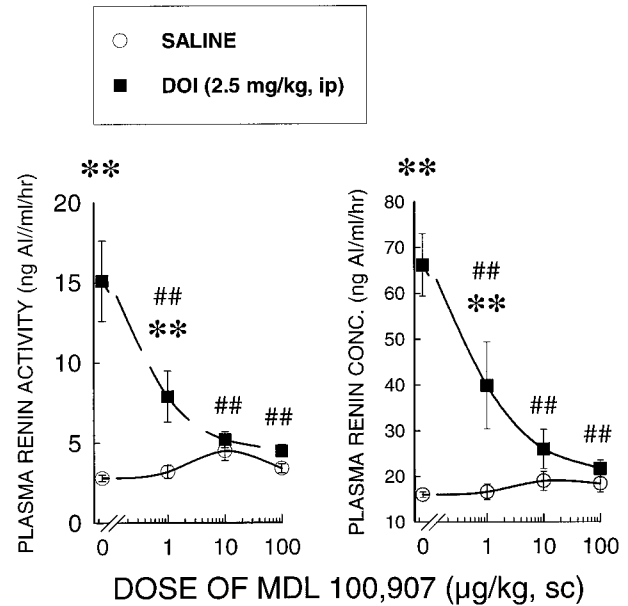


Figure 3. The effect of increasing doses of MDL 100,907 on plasma renin activity (left) and concentration (right) in rats challenged with either saline or DOI (2.5 mg/kg, i.p.). Circles represent rats challenged with saline, whereas squares represent rats challenged with DOI (2.5 mg/kg, i.p.). The data represent the mean \pm SEM of eight rats per group. **Significant effect of DOI, $p < 0.01$; ## significant effect of MDL 100,907, $p < 0.01$ (Newman–Keuls multiple range test).

were all significant. The Newman–Keuls multiple range test revealed that the lowest dose of MDL 100,907 (0.001 mg/kg, s.c.) significantly inhibited the effect of DOI on plasma ACTH levels (by 58%). A similar effect of this dose of MDL 100,907 was noted for the corticosterone response to DOI (46% reduction).

For oxytocin, the two-way ANOVA revealed a significant main effect of MDL 100,907 ($F_{(3,53)} = 10.050$; $p < 0.01$), a significant main effect of DOI ($F_{(1,53)} = 20.21$; $p < 0.01$), and a significant interaction between MDL 100,907 and DOI ($F_{(3,53)} = 7.92$; $p < 0.01$). For prolactin, the two-way ANOVA revealed a main effect of MDL 100,907 ($F_{(3,50)} = 8.48$; $p < 0.01$), main effect of DOI ($F_{(1,50)} = 17.21$; $p < 0.01$), and interaction between MDL 100,907 and DOI ($F_{(3,50)} = 8.43$; $p < 0.01$) that were all statistically significant. The Newman–Keuls multiple range test revealed that the subcutaneous 0.001 mg/kg dose of MDL 100,907 inhibited the effect of DOI on plasma oxytocin levels (by 57%). An even more dramatic effect of this dose of MDL 199,907 was seen in the prolactin response to DOI (74% reduction).

For plasma renin activity, the two-way ANOVA revealed a significant main effect of MDL 100,907 ($F_{(3,54)} = 10.42$; $p < 0.01$), a significant main effect of DOI ($F_{(1,54)} = 48.79$; $p < 0.01$), and a significant interaction between MDL 100,907 and DOI ($F_{(3,54)} = 15.69$; $p < 0.01$). The two-way ANOVA for plasma renin concentration was similar: main effect of MDL 100,907 ($F_{(3,54)} = 9.01$; $p < 0.01$), main effect of DOI ($F_{(1,54)} = 45.01$; $p < 0.01$), and an interaction between MDL 100,907 and DOI ($F_{(3,54)} = 11.68$; $p < 0.01$), which were statistically significant. Finally, the Newman–Keuls test revealed that the lowest dose of MDL 100,907 (0.001 mg/kg, s.c.) produced significant reductions in the effect of DOI on plasma renin activity (48%) and plasma renin concentration (40%).

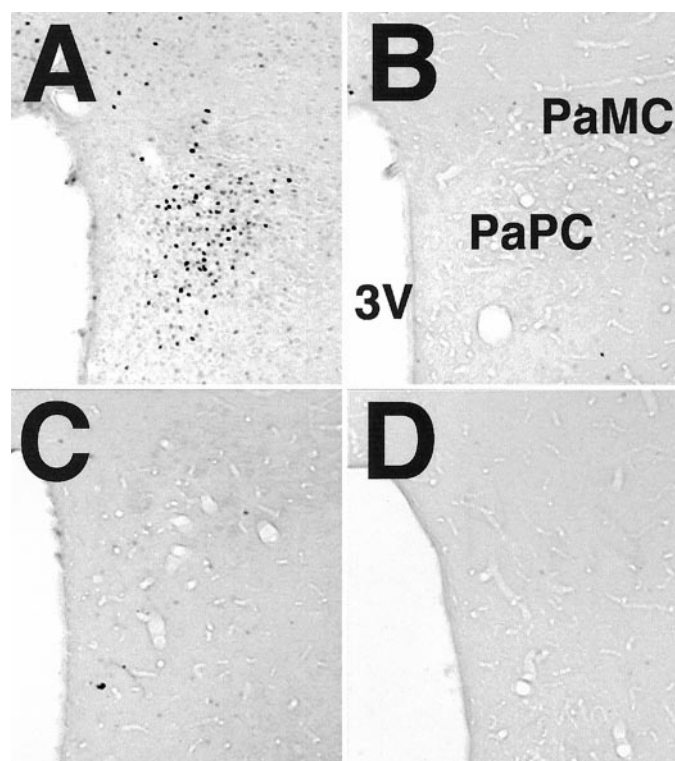


Figure 4. Photomicrographs illustrating DOI-induced increase in Fos immunoreactivity in cells of the paraventricular nucleus of the hypothalamus and its blockade by pretreatment with MDL 100,907. *A*, Saline pretreated rats challenged with DOI (2.5 mg/kg, i.p.). *B*, Rats that were pretreated with MDL 100,907 (0.1 mg/kg, s.c.) and subsequently challenged with DOI (2.5 mg/kg, i.p.). *C*, Rats that were pretreated with MDL 100,907 (0.1 mg/kg, s.c.) and subsequently received a saline injection. *D*, Rats that were pretreated with saline and received an injection of saline. *3V*, Third ventricle; *PaMC*, magnocellular paraventricular hypothalamic nucleus; *PaPC*, parvocellular paraventricular hypothalamic nucleus.

Experiment 2: Fos study

DOI-induced Fos immunoreactivity was observed in the following hypothalamic regions: suprachiasmatic, anterior hypothalamic, paraventricular, supraoptic, dorsomedial, ventromedial, and arcuate nuclei. A particularly high density of DOI-induced Fos-labeled neurons was seen in the parvocellular part of the paraventricular hypothalamic nucleus (Fig. 4), but a scattering of Fos labeling was consistently observed in all other subdivisions of this nucleus. Fewer neurons were found scattered within the supraoptic nucleus. In animals treated with DOI, MDL 100,907 (0.1 mg/kg, s.c.) pretreatment clearly decreased Fos immunostaining in all hypothalamic regions examined. This was especially apparent in the hypothalamic paraventricular nucleus (Fig. 4). Rats treated with MDL 100,907 plus DOI also did not differ in their staining (Fig. 4) from rats treated with saline or MDL 100,907 alone. In the paraventricular nucleus and supraoptic nucleus, there was a significant difference between the group of DOI-treated rats and all other groups (Table 1). The two-way ANOVA for Fos immunoreactivity in the paraventricular nucleus indicated a significant main effect of DOI ($F_{(1,12)} = 16.53$; $p < 0.01$) and a significant interaction between MDL 100,907 and DOI ($F_{(1,12)} = 42.24$; $p < 0.01$). Similarly, in the supraoptic nucleus, the two-way ANOVA for Fos immunoreactivity indicated a significant main effect of DOI ($F_{(1,12)} = 16.51$; $p < 0.01$) and a significant interaction between MDL 100,907 and DOI

Table 1. DOI-induced increase in Fos immunoreactivity (Fos-IR) in hypothalamic nuclei and its blockade by pretreatment with the 5-HT_{2A} antagonist MDL 100,907

Treatment	Number of Fos-IR cells in hypothalamic nuclei	
	Paraventricular	Supraoptic
Saline–saline	59.75 ± 23.5	8.5 ± 3.1
Saline–DOI	575.25 ± 60.8**	26.0 ± 2.2**
MDL 100,907–saline	165.25 ± 50.9	6.0 ± 0.7
MDL 100,907–DOI	117.0 ± 26.1	3.5 ± 2.2

The data represent the mean ± SEM of four rats per group. MDL 100,907 (0.1 mg/kg, s.c.) was injected 45 min before DOI (2.5 mg/kg, i.p.). The rats were killed 2 hr after the DOI injection.

**Significant difference from all other groups; $p < 0.01$ (two-way ANOVA and Newman–Keuls multiple range test).

($F_{(1,12)} = 10.57$; $p < 0.01$). The Newman–Keuls test for both nuclei indicated that the rats receiving DOI were significantly different from all other groups ($p < 0.01$). Hence, MDL 100,907 significantly reduced DOI-induced Fos immunoreactivity (Table 1).

Dual label immunohistochemistry revealed that most CRF-containing cells in the parvocellular part of the paraventricular nucleus contained Fos immunoreactivity (Fig. 5). However, there were many Fos-immunostained neurons that were not immunoreactive to CRF. Several oxytocin cells in the paraventricular nucleus and supraoptic nucleus also contained Fos immunoreactivity (Fig. 5). All vasopressin immunoreactive neurons were devoid of Fos immunostaining in animals treated with DOI (Fig. 5). MDL 100,907 (0.1 mg/kg, s.c.) significantly reduced DOI-induced Fos immunoreactivity in the paraventricular nucleus (Fig. 4), supraoptic nucleus, and other hypothalamic regions including those containing the CRF and oxytocin neurons.

Fos immunoreactivity was also found consistently in many other areas of the forebrain. A distinct pattern of Fos immunoreactivity was observed in the caudolateral subdivisions of the central nucleus of the amygdala (Fig. 6*A*) and dorsal parts of the lateral bed nucleus of the stria terminalis. Fos immunoreactivity also was observed in the cortical amygdaloid nucleus and surrounding cortical regions. Fos-labeled cells were observed as well in the following areas: prefrontal cortex (prelimbic, infralimbic, anterior cingulate, etc.), thalamus, and olfactory bulb. MDL 100,907 blocked the effects of DOI. When administered alone, MDL 100,907 did not induce any increases in Fos immunoreactivity compared with saline-treated controls.

Dual label immunohistochemistry revealed DOI-induced Fos immunoreactivity in enkephalin-expressing neurons in both the amygdala and bed nucleus of the stria terminalis. Fos immunoreactivity was not observed in CRF immunoreactive neurons in the amygdala (Fig. 6*B*), in cortex, and bed nucleus of the stria terminalis. Fos immunoreactivity in cortex, amygdala, and bed nucleus of the stria terminalis was not observed in rats pretreated with MDL 100,907, suggesting that the Fos induced in these areas by DOI treatment is mediated by 5HT_{2A} receptor activation.

DISCUSSION

This study suggests that 5-HT_{2A} receptor activation induces a characteristic release of hormones and a concurrent pattern of Fos immunoreactivity in the brain. The data suggest that the CRF and oxytocin neurons in the hypothalamic paraventricular nucleus mediate the ACTH and oxytocin responses to activation of 5-HT_{2A} receptors. It is not clear whether these neurons also

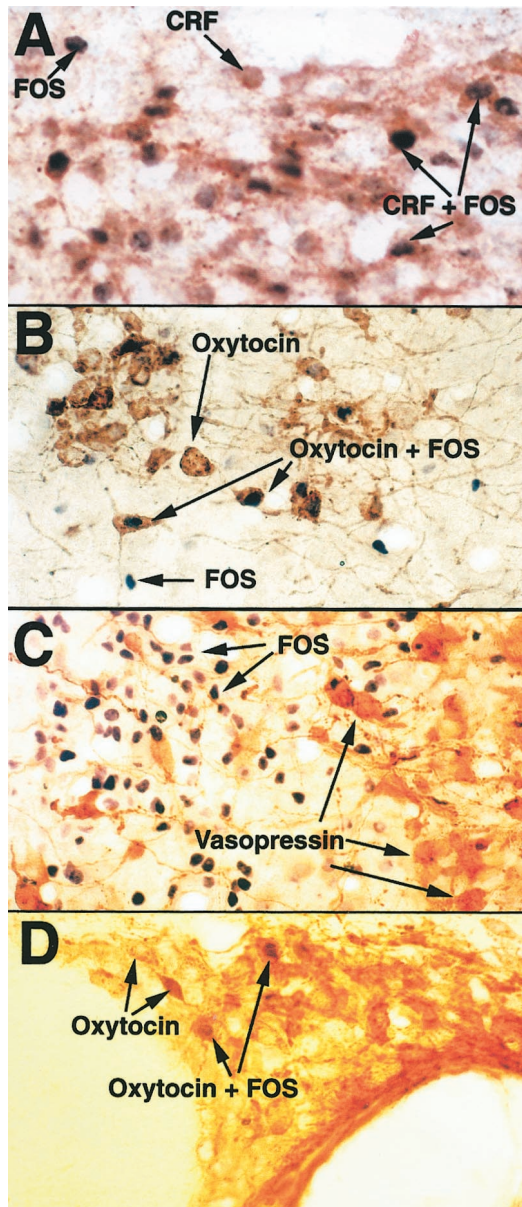


Figure 5. Double label immunohistochemical labeling photomicrographs illustrating DOI-induced Fos immunoreactivity in the hypothalamus. *A*, CRF and Fos in the paraventricular nucleus. *B*, Oxytocin and Fos in the paraventricular nucleus. *C*, Vasopressin and Fos in the paraventricular nucleus. *D*, Oxytocin and Fos in the supraoptic nucleus. The rats received an injection of DOI (2.5 mg/kg, i.p.) 2 hr before killing.

mediate the effects of DOI on the secretion of prolactin and renin. Other neurons in the hypothalamic paraventricular nucleus could mediate the effects of DOI on the secretion of prolactin and renin (see below).

DOI, the most selective 5-HT_{2A/2C} agonist available to date (compared with its affinity for other receptors), has a similar affinity for 5-HT_{2A} and 5-HT_{2C} receptors (Hoyer, 1988; Van Wijngaarden et al., 1990). DOI has been used to examine the role of 5-HT_{2A/2C} receptors in several physiological and behavioral phenomena such as temperature control, increased blood pressure and heart rate, increased secretion of hormones, wet dog shakes, ear scratching, and feeding (McCall and Harris, 1988; Schechter and Simansky, 1988; Bagdy et al., 1989; Dabiré et al.,

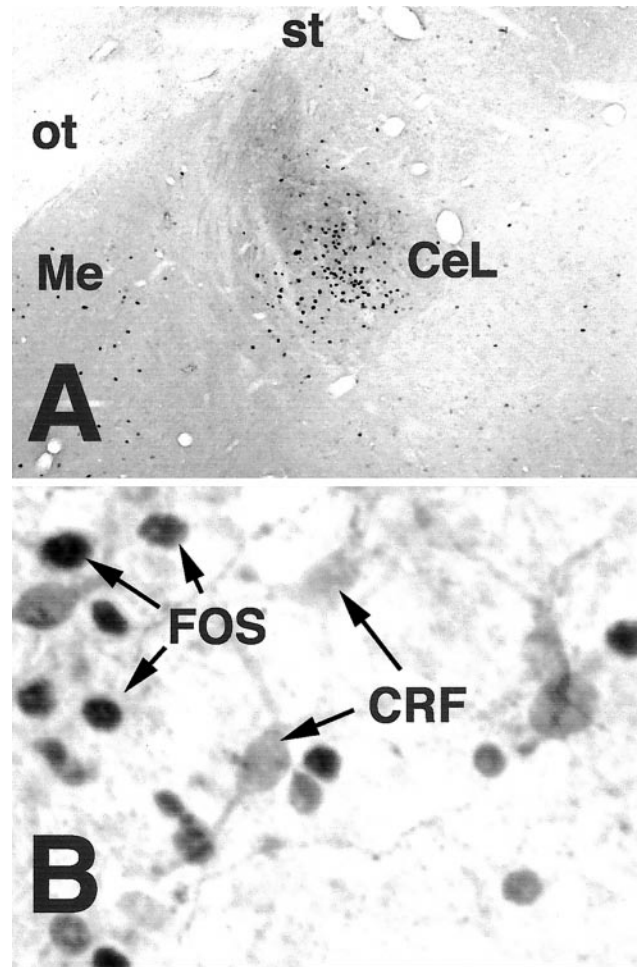


Figure 6. Dual label immunohistochemical photomicrographs illustrating CRF- and DOI-induced Fos immunoreactivity and CRF in the amygdala. *A*, Low-power photomicrograph illustrating the location of CRF and Fos labeling in the lateral part of the central nucleus of the amygdala (CeL). *B*, High-power photomicrograph of the same region illustrating CRF immunoreactive neuron (cytoplasmic stain) and Fos-immunostained cells (nuclear stain). There are no neurons that contain both CRF and Fos immunoreactivity. *ot*, Optic tract; *st*, stria terminalis; *Me*, medial amygdaloid nucleus.

1989; Alper, 1990a,b; Chaouloff et al., 1990; Darmani et al., 1990a,b; Kozuru et al., 2000). In many of these studies, it was difficult to determine whether 5-HT_{2A} or 5-HT_{2C} receptors mediate these effects of DOI because the three main antagonists that were available, ketanserin, ritanserin, and spiperone, are not sufficiently selective. For example, ketanserin, the standard 5-HT₂ antagonist, has a modest affinity for α -noradrenergic receptors and only has a 10-fold difference in affinity between 5-HT_{2A} and 5-HT_{2C} receptors (Van Wijngaarden et al., 1990). Ritanserin has approximately the same affinity for 5-HT_{2A} and 5-HT_{2C} receptors, and spiperone, which has an \sim 100-fold difference in affinity between 5-HT_{2A} and 5-HT_{2C} receptors, is a powerful dopamine D₂ antagonist (Leysen et al., 1985; Roth et al., 1994; Khorana et al., 1999). As mentioned in the introductory remarks, this high affinity of spiperone for dopamine D₂ receptors is a problem for studies examining the secretion of prolactin. Hence, the introduction of MDL 100,907 (with a >100-fold higher affinity for 5-HT_{2A} than for 5-HT_{2C} receptors and low affinity for dopamine D₂ receptors) (Schreiber et al., 1995; Kehne et al., 1996) enabled us

to examine the relative importance of 5-HT_{2A} and 5-HT_{2C} receptors in the regulation of all hormones.

A wide range of hormones was selected in our study to represent different levels of regulation. Oxytocin is the most direct marker of events occurring inside the brain because it is secreted into the circulation from the pituitary neural lobe from nerve terminals of neurons originating inside the hypothalamus. By comparison, ACTH and prolactin represent amplification of the signal because their secretion is dependent on hypothalamic releasing and/or inhibiting hormones that are transported to the pituitary gland and activate G-protein-linked receptors to initiate the activation of corticotrophs and lactotrophs. Corticosterone represents an even greater level amplification because ACTH that is released from the pituitary activates specific ACTH receptors (which also couple to G-proteins) in the adrenal cortex and activates the secretion of corticosterone. Finally, renin represents an endocrine system that is centered in the kidneys and is regulated by a complex system that includes the hypothalamic paraventricular nucleus, sympathetic nervous system, and a hypothalamic renin-releasing factor (Van de Kar et al., 1987; Van de Kar and Blair, 1999). In the present study, DOI-induced secretion of all these hormones was prevented by very low doses of the 5-HT_{2A} antagonist MDL 100,907. This remarkable similarity of the response of all these neuroendocrine systems to the same doses of both DOI and MDL 100,907 suggests that the same 5-HT_{2A} receptor mechanism is involved. Thus, *in vivo*, DOI may be a more effective 5-HT_{2A} agonist than a 5-HT_{2C} agonist. Other studies agree with this conclusion, because the effects of DOI but not of a 5-HT_{2C} agonist, m-chlorophenyl piperazine, on plasma ACTH and prolactin levels can be inhibited by another 5-HT_{2A} antagonist, ketanserin (Bagdy, 1996).

Studies using electrophysiological recording and lesion approaches indicate that the hypothalamic paraventricular nucleus is crucial for the serotonergic stimulation of ACTH, corticosterone, oxytocin, prolactin, and renin secretion (Gotoh et al., 1987; Liposits et al., 1987; Saphier, 1991; Kawano et al., 1992; Rittenhouse et al., 1992, 1993, 1994; Bagdy and Makara, 1994; Van de Kar et al., 1995). Specifically, mechanical destruction of the hypothalamic paraventricular nucleus prevents the DOI-induced increase in plasma levels of oxytocin, ACTH, corticosterone, and prolactin (Bagdy, 1996). Although no immunocytochemical evidence exists for the colocalization of 5-HT_{2A} receptors in CRF or oxytocin cells in the hypothalamus, there is autoradiographic evidence for the presence of 5-HT_{2A} receptors in the hypothalamic paraventricular nucleus (Appel et al., 1990). Furthermore, *in situ* hybridization studies also indicate that mRNA coding for 5-HT_{2A} receptors can be detected in the hypothalamic paraventricular nucleus (Wright et al., 1995; Gundlach et al., 1999). Therefore, it is likely that the effect of DOI on the secretion of ACTH, corticosterone, oxytocin, prolactin, and renin is mediated by activation of 5-HT_{2A} receptors in hypothalamic paraventricular neurons. However, because it is not certain that these 5-HT_{2A} receptors are colocalized on CRF or oxytocin neurons, these effects of DOI could be mediated via an interneuron. An example of such an interneuronal interaction was demonstrated in the cortex (Mackowiak et al., 1999).

A previous study reported that administration of the same dose of DOI (2.5 mg/kg, i.p.) to rats increases Fos expression in the hypothalamic paraventricular nucleus (Rouillard et al., 1996). In the present study, we observed that the DOI-induced expression of Fos immunoreactivity occurs in CRF- and oxytocin-containing neurons of the hypothalamic paraventricular nucleus. Moreover,

we observed that this phenomenon is exclusively mediated by activation of 5-HT_{2A} receptors because it is blocked by a low dose (0.1 mg/kg, s.c.) of MDL 100,907. The neuroanatomical evidence indicating that administration of DOI induces Fos expression in CRF and oxytocin neurons in the hypothalamic paraventricular nucleus agrees with the hypothesis that activation of 5-HT_{2A} receptors in these neurons stimulates them to increase the secretion of ACTH and oxytocin, respectively.

Our findings regarding the anatomical distribution of Fos immunoreactivity are in good agreement with previous studies using DOI and Fos as a marker for neuronal activation (Leslie et al., 1993a; Rouillard et al., 1996; Tilakaratne and Friedman, 1996). These previous studies reported DOI-induced Fos expression in the following areas: frontal, parietal, cingulate and piriform cortical regions, hypothalamus, bed nucleus of the stria terminalis, amygdala, striatum, thalamus, mammillary bodies, globus pallidus, and hippocampus. However our findings differ from the above studies in that we also observed staining in the septum and in the choroid plexus. We did not observe staining in the mammillary bodies. However, our sampling of brain sections did not extend caudal enough to include the mammillary bodies. Finally, pretreatment with MDL 100,907 blocked DOI-induced staining in all of the above regions that we reported except for the choroid plexus, where there is a high density of 5-HT_{2C} receptors. Thus, our findings suggest an extensive distribution of Fos immunoreactive neurons in the forebrain that are selectively activated via the 5-HT_{2A} properties of DOI.

Changes in 5-HT_{2A} receptors have been associated with both cognitive and mood disorders. Evidence supporting such a role for 5-HT_{2A} receptors has come from studies using several approaches, such as PET scanning, ligand binding studies in brain tissues obtained postmortem from suicide victims, and polymorphism of genes encoding the 5-HT_{2A} receptors (Massou et al., 1997; Meltzer, 1999; Meyer et al., 1999; Aubert et al., 2000; Du et al., 2000). Although most of these studies examine factors controlling the density of 5-HT_{2A} receptors, very little information can be obtained about the functioning of the whole 5-HT_{2A} receptor-signaling system in humans. The agonist-induced increase in plasma levels of hormones represents an activation of the whole 5-HT_{2A} receptor-signaling cascade in the hypothalamus. Because of its hallucinogenic properties, DOI cannot be administered to humans. However, the results of the present study may stimulate the development of 5-HT_{2A} agonists that can be used in human neuroendocrine challenge tests. Thus, the results of our studies may provide a neuroanatomical foundation for examining neuroendocrine responses to specific 5-HT agonists as a functional peripheral marker of the functioning of the whole 5-HT_{2A} receptor system in humans.

In conclusion, the present study provides neuroanatomical and pharmacological data suggesting that activation of 5-HT_{2A} receptors on CRF and oxytocin neurons in the hypothalamic paraventricular nucleus stimulates the secretion of ACTH and oxytocin. A parallel DOI-induced activation of enkephalin neurons in the amygdala and bed nucleus of the stria terminalis also is mediated by 5-HT_{2A} receptors. Hence, it is possible that the neuroendocrine response to 5-HT_{2A} agonists may be useful as a peripheral marker of the functioning of 5-HT_{2A} receptors in the hypothalamus and other limbic brain regions. The information obtained in this study can provide a neuroanatomical and neurochemical foundation that may lead to neuroendocrine challenge tests in humans suffering from mood disorders to examine the possible alterations in 5-HT_{2A} receptor systems in their brain.

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