

## **Neuroendocrine Evidence That Ro 60-0175 Is Not a Selective 5-HT<sub>2C</sub> Receptor Agonist\***

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## Abstract

The serotonin 2A and 2C (5-HT<sub>2A</sub> and 5-HT<sub>2C</sub>) receptors are so closely related that selective agonists have not been developed until recently with the advent of Ro 60-0175, a putatively selective 5-HT<sub>2C</sub> receptor agonist. In the present study, Ro 60-0175 was used to analyze the importance of 5-HT<sub>2C</sub> receptors in hormone secretion. Injection of Ro 60-0175 (5 mg/kg, s.c.) produced a maximum increase in plasma levels of adrenocorticotrophic hormone (ACTH), oxytocin, and prolactin at 15 minutes post-injection and a maximum increase in plasma corticosterone levels at 60 minutes post-injection. Ro 60-0175-mediated increases in plasma hormone levels were dose-dependent (corticosterone ED<sub>50</sub> = 2.43 mg/kg; oxytocin ED<sub>50</sub> = 4.19 mg/kg; prolactin ED<sub>50</sub> = 4.03 mg/kg). To assess the role of 5-HT<sub>2C</sub> and 5-HT<sub>2A</sub> receptors in mediating the hormone responses to Ro 60-0175, rats were pretreated with the 5-HT<sub>2C</sub> antagonist SB 242084 or 5-HT<sub>2A</sub> antagonists MDL 100,907 prior to injection of Ro 60-0175 (5 mg/kg, s.c.). Neither SB 242084 (0.1, 0.5, 1, 5 mg/kg, i.p.) nor MDL 100,907 (1, 5, 10 µg/kg, s.c.) significantly inhibited the Ro 60-0175-induced increases in plasma hormone levels. The data suggest that Ro 60-0175 increases hormone secretion by mechanisms independent of the activation of 5-HT<sub>2C</sub> and/or 5-HT<sub>2A</sub> receptors and suggest that Ro 60-0175 is not a highly selective 5-HT<sub>2C</sub> receptor agonist.

Keywords: Ro 60-0175, ACTH, corticosterone, oxytocin, prolactin, 5-HT<sub>2A</sub> receptor

Serotonin<sub>2</sub> (5-HT<sub>2A</sub> and/or 5-HT<sub>2C</sub>) receptors play important roles in depression, obsessive compulsive disorder, eating disorders, and schizophrenia (Blier and de Montigny, 1999; Aghajanian and Marek, 2000). Both 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors are G-protein linked receptors that couple to phospholipase C (PLC) as a second messenger and thereby increase diacylglycerol, inositol trisphosphate and intracellular Ca<sup>2+</sup> levels (reviewed by Barnes and Sharp, 1999). The lack of selective agonists for each 5-HT<sub>2</sub> receptor subtype has hindered a precise differentiation between 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor-mediated effects.

The involvement of the 5-HT<sub>2C</sub> and/or 5-HT<sub>2A</sub> receptors in the regulation of hormone secretion has been examined with a variety of 5-HT<sub>2</sub> receptor agonists, the most common being (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane HCl (DOI). DOI increases the plasma levels of adrenocorticotrophic hormone (ACTH), corticosterone, oxytocin, prolactin, and renin (Rittenhouse et al., 1993; Rittenhouse et al., 1994; Van de Kar et al., 2001). DOI was shown to stimulate the secretion of ACTH, corticosterone, oxytocin, prolactin, and renin by specifically activating the 5-HT<sub>2A</sub> receptor as the increase in plasma levels of all these hormones was blocked by very low doses of the 5-HT<sub>2A</sub> receptor antagonist MDL 100,907 (Van de Kar et al., 2001). Moreover, evidence suggests that 5-HT<sub>2A</sub> receptors mediate hormone responses to other 5-HT<sub>2C/2A</sub> agonists, such as m-CPP, MK-212 and quipazine. The increase in plasma corticosterone levels after injection of MK-212, m-CPP and quipazine are blocked by MDL 100,907. On the other hand, SB 242084 and SB 200646A, 5-HT<sub>2C</sub> receptor antagonists, do not inhibit the m-CPP, MK-212 and quipazine-mediated increases in plasma corticosterone levels (Hemrick-Luecke and Fuller, 1996; Hemrick-Luecke and Evans, 2002). Both studies

provide evidence for the importance of the 5-HT<sub>2A</sub> receptor subtype in the regulation of neuroendocrine responses. However, there is no direct evidence to evaluate the role of the 5-HT<sub>2C</sub> receptor subtype in the secretion of ACTH, oxytocin, or prolactin. Thus, we used Ro 60-0175, an agonist with a high affinity for 5-HT<sub>2C</sub> receptors, in an attempt to determine the role of 5-HT<sub>2C</sub> receptors in hormone secretion.

Ro 60-0175 has a high affinity for human recombinant 5-HT<sub>2C</sub> receptors ( $pK_i = 9.0$ ) and 5-HT<sub>2B</sub> receptors ( $pK_i = 9.3$ ), a lower affinity for 5-HT<sub>2A</sub> receptors ( $pK_i = 7.5$ ), and an even lower affinity for other serotonin receptors (5-HT<sub>1A</sub>,  $pK_i = 5.4$ ; 5-HT<sub>1B</sub>,  $pK_i = 5.3$ ; and 5-HT<sub>3</sub>,  $pK_i = 5.2$ ) (Martin et al., 1998; Cussac et al., 2002). The affinity of Ro 60-0175 for non-serotonergic receptors has not been documented. Ro 60-0175 has been widely used in behavioral studies and shown to be 5-HT<sub>2C</sub> receptor selective because the 5-HT<sub>2C</sub> receptor antagonist SB 242084 blocked its effects on hypolocomotion, penile grooming, and as a discriminative stimulus (Dekeyne et al., 1999; Higgins et al., 2001). The doses of Ro 60-0175, in the present study, were chosen based on published data showing changes in behavioral responses and in neurotransmitter release (Martin et al., 1998; Millan et al., 1998).

SB 242084 is a selective 5-HT<sub>2C</sub> receptor antagonist ( $pK_i = 9.0$ ), as its affinity for other serotonergic and non-serotonergic receptors is considerably lower (5-HT<sub>2B</sub>,  $pK_i = 7.0$ ; 5-HT<sub>2A</sub>,  $pK_i = 6.8$ ;  $\alpha_1$ -adrenergic,  $pK_i < 5.0$ ) (Kennett et al., 1997). The doses of SB 242084 were chosen based on published data showing inhibition of Ro 60-0175-mediated behavior (hypoactivity, penile grooming) and neurotransmitter release (Millan et al., 1998; Higgins et al., 2001).

MDL 100,907 is a selective 5-HT<sub>2A</sub> receptor antagonist ( $pK_i = 9.07$ ). Its affinity for other receptors is separated by at least one log scale (5-HT<sub>2C</sub>,  $pK_i = 7.06$ ;  $\alpha_1$ -adrenergic,  $pK_i = 6.89$ ) (Kehne et al., 1996). The doses of MDL 100,907 used were based on their ability to block DOI-mediated increases in hormone levels and to avoid occupancy of the 5-HT<sub>2C</sub> receptor (Smith et al., 1999; Van de Kar et al., 2001).

In the present study, we used the 5-HT<sub>2A</sub> receptor antagonist MDL 100,907 and the selective 5-HT<sub>2C</sub> antagonist SB 242084 in conjunction with Ro 60-0175 to determine the importance of the 5-HT<sub>2A</sub> and/or 5-HT<sub>2C</sub> receptor in Ro 60-0175-mediated hormone secretion.

## Methods

### *Animals*

Male Sprague-Dawley rats (225-275 g) were purchased from Harlan Laboratories (Indianapolis, IN, U.S.A.). The animals were housed two per cage in an environment controlled for lighting (7 AM-7 PM), temperature, and humidity. Food and water were available ad libitum. Eight to ten 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 the Loyola University Institutional Animal Care and Use Committee (IACUC).

### *Drugs*

Ro 60-0175 ((S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine fumarate) was donated by Hoffman-La Roche Ltd. (Basel, Switzerland). MDL 100,907 ((±) 2, 3-dimethoxyphenyl-1-[2-4-(piperidine)-methanol]) was donated by Hoechst Marion Roussel Research Institute (Cincinnati, OH, U.S.A.). SB 242084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbonyl] indoline) was donated by SmithKline Beecham Pharmaceuticals (Harlow, UK). The solubilizing agent, 2-hydroxypropyl-β-cyclodextrin (HBC), was purchased from Sigma/RBI (Saint Louis, MO, U.S.A.).

Ro 60-0175 was injected at doses of 0.1, 0.5, 2.5, 5, and 10 mg/kg (in a volume of 2 ml/kg, s.c.). It was dissolved directly in 0.9% saline. MDL 100,907 was injected at doses of 1, 5, and 10 µg/kg (1 ml/kg, s.c.). MDL 100,907 was dissolved in a minimum volume of 0.01 N HCl and further diluted to its final concentration using 0.9% saline. SB 242084 was injected at doses of 0.1, 0.5, 1, 5 mg/kg (2 ml/kg, i.p.). SB 242084 was

dissolved in 0.9% saline containing 8% 2-hydroxypropyl- $\beta$ -cyclodextrin (w/v) and 25 mM citric acid, heated with constant stirring, and allowed to cool to room temperature.

### ***Experimental Procedures***

The rats were handled for 4 days prior to the treatment/sacrifice day. Rats were randomly assigned to the experimental groups (n = 8 to 10); cage mates were assigned to the same experimental groups.

For the time course experiment, Ro 60-0175 was administered at a dose of 5 mg/kg (2 ml/kg, s.c.) 15, 30, 60, and 120 minutes prior to decapitation. Control groups for Ro 60-0175 consisted of 0.9% saline (2 ml/kg, s.c.) administered at 15 and 120 minutes prior to decapitation. Uninjected rats were included within the experimental design to control for injection effects.

For the dose response experiment, Ro 60-0175 was administered at doses of 0.1, 0.5, 2.5, 5, and 10 mg/kg (2 ml/kg, s.c.) 15 minutes before decapitation. Controls received a saline injection (2 ml/kg, s.c.) 15 minutes prior to decapitation.

SB 242084 was injected at doses of 0.1, 0.5, 1, and 5 mg/kg (2 ml/kg, i.p.) 30 minutes before the injection of Ro 60-0175 (5 mg/kg, s.c.). Ro 60-0175 was administered 15 minutes prior to sacrifice. Previous studies indicate that when SB 242084 was administered 45 minutes prior to sacrifice it prevented the ability of Ro 60-0175 to act as a discriminative stimulus (Dekeyne et al., 1999).

MDL 100,907 was administered at doses of 1, 5, and 10  $\mu$ g/kg (1 ml/kg, s.c.) 1 hour and 45 minutes prior to the injection of Ro 60-0175 (5 mg/kg, s.c.). Ro 60-0175 was administered 15 minutes prior to sacrifice.



In all the experiments, the trunk blood was collected in centrifuge tubes containing 0.5 ml of a 0.3 M EDTA (pH 7.4) solution. The plasma samples were stored at -70° C until used for radioimmunoassays.

### ***Radioimmunoassay of Hormones***

Plasma ACTH, corticosterone, oxytocin, and prolactin concentrations were determined by radioimmunoassays as previously described (Li et al., 1993; Li et al., 1997). Briefly, all assays (except for corticosterone) are double antibody assays (utilizing both primary and secondary antibodies). ACTH antiserum was obtained from IgG Corp. (Nashville, TN, U.S.A.) and <sup>125</sup>I-ACTH tracer was obtained from DiaSorin Inc. (Stillwater, MN, U.S.A.). The sensitivity limit is 0.25 pg/tube with intra- and interassay variabilities of 4.2 and 14.6%, respectively. Corticosterone antiserum was obtained from ICN Biomedicals Inc (Costa Mesa, CA, U.S.A.) and <sup>3</sup>H-corticosterone tracer was obtained from NEN Dupont (Boston, MA, U.S.A.). The sensitivity limit is 0.02 ng/tube and the intra- and interassay variabilities are 4.5 and 11.9%, respectively. Rabbit antioxytocin was donated by Dr. Lanny C. Keil (NASA Ames Research Center, Moffat Field, CA, U.S.A.); <sup>125</sup>I-oxytocin tracer was obtained from NEN Dupont (Boston, MA, U.S.A.). The intra- and interassay variabilities are 5 and 9%, respectively. Prolactin antiserum was donated by the NIADDK-NIH and the tracer was prepared using NIADDK rPRL-I-5 and <sup>125</sup>I from NEN Dupont (Boston, MA, U.S.A.). The sensitivity limit is 30 pg/tube with intra-assay variability of 6.8%. All the assays (except for corticosterone) utilized a goat anti-rabbit secondary antibody from CalBioChem (San Diego, CA, U.S.A.).

## ***Statistical Analyses***

All data are presented as the group mean ( $n = 8$  to  $10$ )  $\pm$  SEM. Hormone data for the Ro 60-0175 time-course and dose-response experiments were analyzed by one-way analyses of variance (ANOVA). A two-way ANOVA was used for the SB 242084 and MDL 100,907 experiments. Newman-Keuls' multiple range tests were used to compare group means (Steel and Torrie, 1960). GB-STAT software (Dynamic Microsystems, Inc., Silver Spring, MD, U.S.A.) was used for all statistical analyses. Significant differences were defined as  $p \leq 0.05$ .  $ED_{50}$  values were calculated using nonlinear regression analysis from the dose-response curves with Prism software (GraphPad Software, Inc., San Diego, CA, U.S.A.).

## **Results**

### ***Ro 60-0175 Time Course***

(Figure 1)

Injection of Ro 60-0175 (5.0 mg/kg, s.c.) produced maximal increases in plasma levels of oxytocin (1593 % above basal;  $F_{(6,57)} = 42.31$ ,  $p < 0.0001$ ) and prolactin (641 % above basal;  $F_{(6,57)} = 34.36$ ,  $p < 0.0001$ ) 15 minutes post injection. A maximal increase in plasma ACTH (1412 % above basal;  $F_{(6,57)} = 29.89$ ,  $p < 0.0001$ ) was observed between 15 and 30 minutes post injection, and statistical analysis did not reveal a difference between these two time points ( $p = 0.49$ ) (Figs. 1A, C, D). Maximum plasma corticosterone levels (2876 % above basal) were observed 60 minutes post Ro 60-0175 injection but were significantly elevated above basal (uninjected) levels from 15 minutes (1346 % above basal) onward ( $F_{(6,57)} = 99.65$ ,  $p < 0.0001$ ) (Fig. 1B). No significant difference was observed between hormone levels in the saline challenged groups and the

uninjected group, except for corticosterone levels at 15 minutes post-saline injection ( $p < 0.01$ ).

### ***Ro 60-0175 Dose Response***

(Figure 2)

Administration of Ro 60-0175 produced a dose-dependent increase in plasma levels of ACTH ( $F_{(5,46)} = 31.88$ ,  $p < 0.0001$ ), corticosterone ( $F_{(5,46)} = 20.76$ ,  $p < 0.0001$ ), oxytocin ( $F_{(5,46)} = 47.23$ ,  $p < 0.0001$ ), and prolactin ( $F_{(5,47)} = 7.72$ ,  $p < 0.0001$ ) (Figs. 2A-D). Ro 60-0175, at a dose of 5.0 mg/kg, produced a near maximal effect for corticosterone, oxytocin, and prolactin. Values for the dose that causes 50% of the maximal effect ( $ED_{50}$ ) were calculated for corticosterone ( $ED_{50} = 2.43$  mg/kg), oxytocin ( $ED_{50} = 4.19$  mg/kg), and prolactin ( $ED_{50} = 4.03$  mg/kg). A maximal effect ( $E_{max}$ ) for ACTH was not achieved and therefore an  $ED_{50}$  could not be calculated (Fig. 2A).

### ***Pretreatment With the 5-HT<sub>2C</sub> Receptor Antagonist SB 242084***

(Figure 3)

Administration of SB 242084 alone did not affect the plasma levels of ACTH, corticosterone, oxytocin, or prolactin. Ro 60-0175 (5.0 mg/kg, s.c.), as previously shown, increased plasma levels of ACTH, corticosterone, oxytocin, and prolactin. Pretreatment with SB 242084 (0.1, 0.5, 1, 5 mg/kg, i.p.) did not inhibit Ro 60-0175-mediated increases in ACTH ( $F_{(4,73)} = 1.37$ ,  $p > 0.05$ ), corticosterone ( $F_{(4,73)} = 0.75$ ,  $p > 0.05$ ), oxytocin ( $F_{(4,73)} = 2.19$ ,  $p > 0.05$ ), or prolactin levels ( $F_{(4,72)} = 0.23$ ,  $p > 0.05$ ) (Figs. 3A-D). While SB 242084 seemed to dose-dependently decrease the Ro 60-0175-mediated increases in oxytocin levels, the statistical analysis (2-way ANOVA) indicated that this was not a statistically significant interaction ( $F_{(4,73)} = 2.19$ ;  $p > 0.05$ ) (Fig. 3C).

### ***Pretreatment With the 5-HT<sub>2A</sub> Receptor Antagonist MDL 100,907***

(Figure 4)

Administration of MDL 100,907 alone did not alter plasma hormone levels in rats. As was observed in the previous experiments, Ro 60-0175 produced increases in plasma levels of ACTH, corticosterone, oxytocin, and prolactin. Pretreatment with MDL 100,907 (1, 5, 10 µg/kg, s.c.), did not block Ro 60-0175-induced increases in plasma ACTH ( $F_{(3,62)} = 1.55$ ,  $p > 0.05$ ), corticosterone ( $F_{(3,62)} = 0.64$ ,  $p > 0.05$ ), oxytocin ( $F_{(3,62)} = 1.31$ ,  $p > 0.05$ ), or prolactin levels ( $F_{(3,59)} = 1.10$ ,  $p > 0.05$ ) (Figs. 4A-D).

### **Discussion**

The present study is the first to demonstrate that Ro 60-0175, a commonly used 5-HT<sub>2C</sub> receptor agonist, has an acute effect on the neuroendocrine system. This study clearly indicates that Ro 60-0175 stimulates ACTH, corticosterone, oxytocin, and prolactin secretion by a mechanism independent of the activation of 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors. Our current data show that Ro 60-0175 dose-dependently increases plasma hormone levels, an effect that is not blocked by the 5-HT<sub>2C</sub> receptor antagonist SB 242084 or the 5-HT<sub>2A</sub> receptor antagonist MDL 100,907.

The reason for testing Ro 60-0175 was that a debate exists regarding the role of 5-HT<sub>2C</sub> receptors in the regulation of hormone release. Therefore, Ro 60-0175 seemed to be a good agonist that would help resolve this question. Previous studies have suggested that another putative 5-HT<sub>2C</sub> receptor agonist, m-chlorophenylpiperazine (m-CPP), stimulates the secretion of several hormones by activating 5-HT<sub>2C</sub> receptors (Bagdy et al., 1989; Aulakh et al., 1992). However, m-CPP is not a selective 5-HT<sub>2C</sub> receptor agonist

(Martin et al., 1998). The effect of m-CPP on ACTH and corticosterone release is blocked by the 5-HT<sub>2A</sub> receptor antagonists ketanserin and MDL 100,907, respectively (Jorgensen et al., 1999; Hemrick-Luecke and Evans, 2002). While 5-HT<sub>2A</sub> receptors were identified by immunohistochemistry within the paraventricular nucleus of the hypothalamus, no immunohistochemical evidence exists for the presence of 5-HT<sub>2C</sub> receptors, although evidence suggests that 5-HT<sub>2C</sub> receptor mRNA is located within this area (Wright et al., 1995; Zhang et al., 2002). Considering the fact that both 5-HT<sub>2C</sub> and 5-HT<sub>2A</sub> receptors are expressed by hypothalamic paraventricular neurons, it was surprising that neither a 5-HT<sub>2C</sub> antagonist, nor a 5-HT<sub>2A</sub> antagonist affected the neuroendocrine responses to Ro 60-0175.

While we are the first to provide a comprehensive overview of the acute effects of Ro 60-0175 on plasma ACTH, oxytocin, and prolactin, a previous study examined the effect of acute Ro 60-0175 administration on plasma corticosterone levels (Hemrick-Luecke and Evans, 2002). In that study, Ro 60-0175-mediated increases in plasma corticosterone levels was completely inhibited by both MDL 100,907 (ED<sub>50</sub> = 0.0126 mg/kg) and SB 242084 (ED<sub>50</sub> = 2.0 mg/kg) (Hemrick-Luecke and Evans, 2002). A few differences in the methodological details between this study and our current studies may explain the differences in the results. Hemrick-Luecke and Evans administered both of the antagonists (SB 242084 and MDL 100,907) 15 minutes prior to the injection of Ro 60-0175, and sacrificed the rats 1 hour after injection of Ro 60-0175. However, as can be seen from Figure 1, only plasma corticosterone levels would remain elevated at one hour after Ro 60-0175 administration; all other hormones would have returned to basal levels. Thus, obtaining blood at 15 minutes post Ro 60-0175 injection was necessary to examine

the response of hormones other than corticosterone. Because corticosterone is regulated by both central and peripheral mechanisms (R.H. Alper, 1990), we cannot eliminate the possibility that the inhibition of Ro 60-0175-mediated increases in corticosterone levels by SB 242084 is mediated by a peripheral mechanism. We also tested a dose of 5 mg/kg (s.c.) of Ro 60-0175 while Hemrick-Luecke and Evans used 3 mg/kg (s.c.). Thus, the higher dose of Ro 60-0175 may have activated other, as yet unidentified receptors, which might not be blocked by MDL 100,907 and SB 242084. However, the doses of MDL 100,907 (1 – 10  $\mu$ g/kg, s.c.) we tested completely blocked the effect of DOI (2.5 mg/kg, i.p.) on plasma levels of ACTH, corticosterone, oxytocin, prolactin, and renin (Van de Kar et al., 2001). Therefore, we are confident that Ro 60-0175 at the dose used (5 mg/kg, s.c.) is exerting its effect by stimulating a mechanism or a receptor other than the 5-HT<sub>2C</sub> or 5-HT<sub>2A</sub> receptors. In the following paragraphs, we will attempt to discuss other probable mechanisms mediating the Ro 60-0175-induced increases in plasma hormone levels.

Studies in CHO-K1 cells transfected with rat 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, or 5-HT<sub>2C</sub> receptors suggest that Ro 60-0175 has the highest potency at 5-HT<sub>2B</sub> receptors (pEC<sub>50</sub> = 8.6) versus 5-HT<sub>2C</sub> (pEC<sub>50</sub> = 7.92) and 5-HT<sub>2A</sub> receptors (pEC<sub>50</sub> = 6.78) (Vickers et al., 2001). The agonist profile of Ro 60-0175 was detected by its ability to increase intracellular calcium levels. A similar rank potency was observed when human 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, or 5-HT<sub>2C</sub> receptors were transfected into the same cell line (Porter et al., 1999). There are several reasons to interpret these data with caution. First, while 5-HT<sub>2B</sub> receptor mRNA has not been detected in rat hypothalamus, its protein expression has been shown to exist in rat dorsal hypothalamic nucleus (Flanigan et al., 1995; Duxon et

al., 1997). No quantitative comparison exists for receptor expression between the 5-HT<sub>2B</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2C</sub> receptors in the hypothalamus or any brain region. The transfected CHO cells likely express higher amounts of 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptors than can be found in the hypothalamus. Also, the importance of 5-HT<sub>2B</sub> receptors within the hypothalamus and in neuroendocrine regulation is unknown. At the present time, we have no access to specific 5-HT<sub>2B</sub> agonists and antagonists to examine whether the effect of Ro 60-0175 on plasma hormone levels is mediated by 5-HT<sub>2B</sub> receptors.

Ro 60-0175 is not likely to increase synaptic concentrations of 5-HT and thereby indiscriminately activate post-synaptic 5-HT receptors mediating hormone secretion. Ro 60-0175 was shown not to increase the extracellular levels of 5-HT in frontal cortex (Millan et al., 1998). The structural similarity of Ro 60-0175 to indolamines allows for the possibility that Ro 60-0175 may act at receptors responsive to indolamines without increasing the synaptic concentration of 5-HT. However, binding studies indicate a low affinity of Ro 60-0175 for 5-HT receptors, other than 5-HT<sub>2C</sub> and 5-HT<sub>2B</sub> receptors (Martin et al., 1998; Cussac et al., 2002).

It also is possible that catecholamines, such as dopamine and norepinephrine, may play a role in the effects of Ro 60-0175 on plasma hormone levels. Dopamine has an inhibitory effect on prolactin secretion but stimulates oxytocin and ACTH secretion (Meller et al., 1991; Borowsky and Kuhn, 1992; Ben Jonathan and Hnasko, 2001). Cortical dopamine levels decrease after administration of Ro 60-0175 (Millan et al., 1998). By suppressing dopamine levels, Ro 60-0175 may be alleviating the dopamine-mediated inhibition of prolactin secretion. This specific dopamine hypothesis may explain what we observe with prolactin but does not offer an explanation as to how

oxytocin and ACTH levels increase. On the other hand, the changes observed in cortical dopamine and norepinephrine levels may not be mirrored within the hypothalamic paraventricular nucleus. For example, an increase in cortical norepinephrine was observed after treatment with SB 242084 (Millan et al., 1998). Norepinephrine stimulates the secretion of ACTH and oxytocin (Feldman and Weidenfeld, 1996). If an increase in norepinephrine was also present within the paraventricular nucleus, we should have observed an increase in plasma ACTH and oxytocin levels after administration of SB 242084. However, we observed no effect of SB 242084 alone on hormone levels. Hence, catecholamines are not likely mediators of the effect of Ro 60-0175 on plasma hormones.

An alternative hypothesis, to explain the effects of Ro 60-0175 on plasma hormone levels may be that Ro 60-0175 activates a non-serotonergic receptor, which in turn elevates plasma hormone levels. While Ro 60-0175 has a high affinity for 5-HT<sub>2C</sub> receptors and a lower affinity for 5-HT<sub>2A</sub> receptors, its affinities for receptors outside the 5-HT receptor family have not been documented (Martin et al., 1998). Ro 60-0175 may directly activate another receptor, such as an adrenergic or histamine receptor, which mediates the increase in hormone secretion. The  $\alpha_1$ -adrenergic receptor antagonist prazosin and  $\beta$ -adrenergic receptor antagonist sotalol attenuate stress-induced increases in plasma ACTH and corticosterone levels (Feldman and Weidenfeld, 1996). Furthermore, catecholaminergic neurons mediate stress-induced oxytocin and corticosterone release (Richardson Morton et al., 1990; Knigge et al., 1999). Histamine (H1) receptors are abundant within the paraventricular nucleus of the hypothalamus and histamine is also known to increase the secretion of ACTH and oxytocin (Kjær et al.,



1992). Thus, the possibility that Ro 60-0175 can stimulate receptors and increase hormone secretion cannot be ignored, particularly since no information is presently available regarding the affinity of Ro 60-0175 for these receptors.

In conclusion, our data indicate that Ro 60-0175 dose-dependently increases plasma hormone levels by a mechanism independent of direct stimulation of either 5-HT<sub>2C</sub> or 5-HT<sub>2A</sub> receptors. Thus, the most probable mechanism through which Ro 60-0175 increases hormone release is activation of other receptors that remain to be identified. Hence, consistent with previous reports (Vickers et al., 2001), Ro 60-0175 is not a selective 5-HT<sub>2C</sub> receptor agonist.

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## Footnotes

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**K.J. Damjanoska**, N.A. Muma, Y. Zhang, D. N. D'Souza, F. Garcia, G.A. Carrasco, G.H. Kindel, K.A. Haskins, M. Shankaran, B.R. Petersen and L.D. Van de Kar. The neuroendocrine effects of Ro 60-0175 are not solely mediated by 5-HT<sub>2C</sub> or 5-HT<sub>2A</sub> receptors. American Society for Pharmacology & Experimental Therapeutics (Great Lakes Chapter) -- 15<sup>th</sup> Annual Meeting, Chicago, IL (2002).

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## Figure Legends

**Figure 1.** Time course (0 – 120 minutes) of the Ro 60-0175-induced increase in plasma levels of ACTH (A), corticosterone (B), oxytocin (C), and prolactin (D). \*\* Significant effect when comparing the Ro 60-0175 injected group (filled circles) to the uninjected control (filled triangle),  $p < 0.01$  (One-Way ANOVA, Newman Keuls multiple range test). †† Significant effect when comparing the saline injected group (open circle) to uninjected group,  $p < 0.01$  (one-way ANOVA, Newman Keuls' multiple range test).

**Figure 2.** Effect of various doses of Ro 60-0175 on plasma levels of ACTH (A), corticosterone (B), oxytocin (C), and prolactin (D). \*\* Significant effect of Ro 60-0175 injected group (filled circles) when compared to saline injected group (open circle at 0 mg/kg),  $p < 0.01$  (one-way ANOVA, Newman Keuls' multiple range test).

**Figure 3.** Effect of SB 242084 pretreatment (0.1, 0.5, 1.0, 5.0 mg/kg, i.p.) on Ro 60-0175 (5.0 mg/kg, s.c.) induced increases in plasma levels of ACTH (A), corticosterone (B), oxytocin (C), and prolactin (D). \*\* Significant effect when comparing the Ro 60-0175 injected group (filled circles) to saline injected group (open circles),  $p < 0.01$  (two-way ANOVA, Newman-Keuls' multiple range test).

**Figure 4.** Effect of MDL 100, 907 pretreatment (1, 5, and 10  $\mu\text{g}/\text{kg}$ , s.c.) on Ro 60-0175 (5.0 mg/kg, s.c.) induced increases in plasma levels of ACTH (A), corticosterone (B), oxytocin (C), and prolactin (D). \*\* Significant effect when comparing the Ro 60-0175

injected group (filled circles) to their respective saline injected group (open circles),  $p < 0.01$  (two-way ANOVA, Newman-Keuls' multiple range test).







