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Lack of effects of 5-HT_3 antagonists on normal and morphine-attenuated sexual behaviours in female and male rats

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Abstract. Although 5-HT_1 and 5-HT_2 receptor activity is known to influence copulation, the effects of 5-HT_3 receptor-selective drugs on sexual activity have yet to be systematically studied. The following experiments investigated the effects of the 5-HT_3-selective antagonists MDL 72222, ondansetron and ICS 205-930 on female sexual behaviour; male rats were studied using ondansetron and granisetron. These compounds influenced neither male nor female copulatory behaviours, suggesting that 5-HT_3 receptors contribute little to the modulation of sexual activity. 5-HT_3 receptor antagonists block certain opioid-induced behaviours and opioids selectively inhibit sexual behaviours; therefore, the ability of ondansetron and ICS 205-930 to modify morphine-attenuated copulatory activity was also tested. While morphine inhibited copulation, 5-HT_3 antagonists failed to reverse the effects.
Key words. Sexual behaviour; serotonin; 5-HT; 5-HT_3 receptors; 5-HT_3 antagonists; opioids; rats.

Although considerable data exist regarding contributions made by 5-HT_1 and 5-HT_2 receptors to the regulation of mammalian sexual activity[1], there are no published studies documenting the influence of 5-HT_3 receptors. In general, serotonergic effects on reproductive behaviours vary with the animal's sex and the subtype of 5-HT receptor activated[1]. In female rats, 5-HT_{1A} receptor activity inhibits, while 5-HT_{1B} and 5-HT_2 receptor activity facilitates, lordosis[1]. In contrast, 5-HT_{1A} receptor activation enhances male rat copulation, while activation of 5-HT_2 or 5-HT_{1B} receptors inhibits such behaviour[1].

The recent identification of brain 5-HT_3 receptors[2] and synthesis of relatively selective 5-HT_3 antagonists has stimulated behavioural pharmacological studies of 5-HT_3 activity. For example, 5-HT_3 antagonists reportedly improve cognitive performance in certain nonhuman species[3,4], attenuate the effects of elevated mesolimbic dopaminergic activity[5,6], act as anxiolytics[7–9], and antiemetics[10] and block certain opiate-induced behaviours[11–13]. (For review of the behavioural pharmacology of 5-HT_3-active compounds, see[14]).

Although no complete studies regarding the effects of 5-HT_3 antagonists on sexual behaviour exist, preliminary findings have been reported. In estrogen-primed female rats, large doses of the selective 5-HT_3 antagonists, ICS 205-930 (5 mg/kg) and MDL 72222 (5 mg/kg) reportedly facilitate, and fail to affect, lordosis, respectively[15]. At dosages of both 0.2 and 0.5 mg/kg, the 5-HT_3 antagonists granisetron (formerly BRL 43694), ondansetron (formerly GR 38032F) and MDL 72222 have been reported to facilitate lordosis in nonreceptive, but not in receptive, female rats[16]. In male rats, ICS 205-930 alone (0.05-5.0 mg/kg) was reportedly without influence on copulatory activity[1]. Together with the known influence of other 5-HT receptor subtypes on sexual behaviour and the existence of 5-HT_3 binding sites in areas fundamental to sexual behaviour such as the hypothalamus[2], these preliminary results suggest that 5-HT_3 receptor activity may influence sexual behaviour.

Evidence suggests a role for endogenous opioids in the regulation of copulatory behaviours[17]; independent of their effectiveness when administered alone, 5-HT_3 receptor antagonists may modulate sexual behaviour via interactions with opioids. At dosages which do not alter gross motoric responses, morphine, a μ-opioid receptor agonist, increases rat hypothalamic 5-HT activity[18] and inhibits copulatory behaviour[17]. 5-HT_3 antagonists antagonize morphine-induced place preference conditioning[11–13], a finding consistent with the possibility that 5-HT_3-active compounds may antagonize the effects of opioids on sexual behaviour. Indeed, preliminary data suggest that ICS 205-930 attenuates morphine-induced inhibition of male copulatory behaviour[1].

The following experiments were conducted with an aim towards increasing understanding of the neurochemical basis of reproductive behaviour and reconciling apparent conflicting findings[15,16]. Furthermore, the employment of 5-HT_3 antagonists as clinical drugs, primarily antiemetics[14], makes full characterization of these compounds essential. The following studies investigated the effects of 5-HT_3 antagonists, administered alone and in conjunction with morphine, on female and male rat sexual behaviour. As functional differences between 5-HT_3 antagonists have been reported[19,20], several such compounds were employed.

Materials and methods

Animals and surgery. Long-Evans rats derived from stock originally obtained from Charles River, Quebec,

served as subjects. Animals were housed in standard laboratory cages in colonies maintained on reverse 12 h/12 h light/dark cycles at 21 ± 1 °C, with food and water available ad libitum.

At a minimum age of 60 days, all female rats underwent bilateral ovariectomy while under general anaesthesia (45 mg/kg sodium pentobarbital and 40 mg/kg ketamine). Surgery occurred at least one week prior to any behavioural testing. Sexual receptivity was induced in the ovariectomized females by s.c. estradiol benzoate (EB) and progesterone (P) (Steraloids) dissolved in 0.1 ml of peanut oil, administered 48 h and 4 h prior to testing, respectively. As female rats showed idiosyncratic responses to these hormones, the dosages necessary to induce appropriate levels of receptivity were established on the basis of pre-drug testing with each group of experimental animals. Nonexperimental stimulus females were primed with 10 µg EB and 500 µg P. s.c. Prior to experimental testing, male rats were screened with stimulus females to ensure copulatory proficiency.

Experimental design. In test series 1 and 2, 5-HT_3 antagonists were systemically administered to ovariectomized female, and male rats, respectively. In test series 3, female and male rats received 5-HT_3 antagonists in conjunction with morphine. Eight experiments, each using a counterbalanced, repeated measures design, were conducted. Subsequent to drug treatment, animals were tested for sexual behaviour. Testing took place at 7 day intervals during the dark phase of the light cycle.

Behavioural scoring was conducted by observers blind to treatment conditions of the animals and occurred while the animals were in clear plexiglas chambers, 45 cm in height and 29 cm in diameter. In studies investigating female behaviours, lordosis quotient (LQ; the number of lordoses in response to 10 mounts by a male × 100%) and the presence or absence of proceptive behaviours (i.e. hopping/darting and ear wiggling) were recorded. In these studies, female rats were introduced into individual chambers and allowed to habituate for 5 min; a stimulus male rat was then placed in the chamber with the female. Testing of female animals continued for 10 min or until 10 mounts had occurred. If a male ejaculated prior to having mounted the female 10 times, he was removed and replaced with a new male. In studies investigating male responses, behaviours scored included: number of mounts and intromissions; latencies to first mount (ML) and to first intromission (IL); ejaculation latency (interval between first intromission and ejaculation); and post-ejaculatory interval (time between ejaculation and first subsequent intromission). In these studies, males were first introduced into the chambers; following a 10 min habituation period, a stimulus female rat was introduced. Thereafter, stimulus female rats were removed and new female rats introduced every 10 min. Testing of males continued for 30 min or until the first post-ejaculatory intromission.

Within individual experiments, each animal received all drug dosages. Proportions of animals displaying ear wiggling and hopping/darting were analyzed via Cochran's Q test, followed by McNemar's test for pairwise comparisons; remaining data were analyzed using Friedman two-way analysis of variance (ANOVA), followed, when appropriate, by Wilcoxon tests for pairwise comparisons. The exception to this occurred in study 3C where each animal received 2 or 3 of 5 possible drug treatments: these data were analyzed via Kruskal-Wallis one-way ANOVA followed by Mann-Whitney tests for pairwise comparisons.

Test series 1 – 5-HT_3 antagonists and female sexual behaviour. MDL 72222 (Research Biochemicals Inc.)[21] and ICS 205-930 (Sandoz)[22] were dissolved in dilute glacial acetic acid and made up to volume with distilled H_2O. Ondansetron (Glaxo)[23] was dissolved in distilled H_2O. All drugs were administered s.c. In study 1A, 15 females primed with 5 µg EB and 100 µg P, received 0 (i.e. vehicle), 0.05, 0.5 or 5 mg/ml/kg MDL 72222 30 min prior to testing. In study 1B, ondansetron was administered to 18 animals primed with 3 µg EB and 50 µg P. Animals received 0 (i.e. vehicle), 0.001, 0.01, 0.1, 1.0, or 10 mg/ml/kg ondansetron 45 min prior to testing. In study 1C, 13 animals received 0 (i.e. vehicle), 0.005, 0.05, 0.5 or 5.0 mg/ml/kg ICS 205-930 40 min prior to testing. In order to replicate the hormonal paradigm employed by Mendelson and Gorzalka[15], animals were primed with EB (7.5 µg) only.

Test series 2 – 5-HT_3 antagonists and male sexual behaviour. Granisetron (Beecham)[24] and ondansetron were dissolved in normal saline and administered SC to 13 and 12 animals, respectively, 30 min prior to testing. In studies 2A and 2B, animals received ondansetron and granisetron, respectively, in the following dosages: 0 (i.e. vehicle), 0.01, 0.1, 1.0, 10.0 and 25.0 mg/ml/kg.

Test series 3 – 5-HT_3 antagonists and morphine. Morphine (BDH Chemicals) was dissolved in distilled H_2O. All drugs were administered s.c. In study 3A, injections of vehicle alone, morphine (2.0 mg/ml/kg) and vehicle, or ondansetron (0.032, 0.16, 0.8 or 4.0 mg/ml/kg) in combination with morphine (2.0 mg/ml/kg) were administered to 18 female rats primed with 3 µg EB and 100 µg P. Ondansetron and morphine were administered 60 and 30 min prior to testing respectively. In study 3B, 12 female rats primed with 7.5 µg EB and 100 µg P received injections of vehicle alone, morphine (2.0 mg/ml/kg) and vehicle, or ICS 205-930 (0.005, 0.05, 0.5 or 5.0 mg/ml/kg) in combination with morphine (2.0 mg/ml/kg). ICS 205-930 and morphine were administered 45 and 30 min prior to testing, respectively. In study 3C ondansetron and morphine were administered to 17 male rats 45 and 30 min prior to testing, respectively. Animals received injections of vehicle alone, morphine (1.5 mg/ml/kg) and vehicle, or ondansetron (0.8, 4.0 or 20.0 mg/ml/kg) in combination with morphine (1.5 mg/ml/kg).

Results and discussion

LQ and IL data are presented in figures 1–4. In test series 1, neither lordotic responses nor proceptive behaviours were affected by the 5-HT_3 antagonists, MDL 72222, ondansetron or ICS 205-930. Similarly, in test series 2, all parameters of male copulatory behaviour were unaffected by administration of either ondansetron or granisetron. In test series 3 morphine profoundly inhibited female (study 3A: $\chi^2 = 23.5$, $p = 0.0003$ LQ; study 3B: $\chi^2 = 15.8$, $p = 0.0073$ LQ) and male sexual activity (study 3C: $\chi^2 = 24.2$, $p = 0.0001$ ML; $\chi^2 = 32.9$, $p < 0.0001$ IL). However, these effects were not attenuated by ondansetron or ICS 205-930.

These findings are congruent with previous data showing MDL 72222 to be ineffective in modulating lordosis[15] and with results indicating that 5-HT_3 antagonists fail to affect sexual behaviour in receptive females[16]. Moreover, the current lack of effect in receptive animals suggests that in nonreceptive animals, previously reported facilitatory effects induced by 5-HT_3 receptor antagonists may partially reflect statistical regression toward the mean, as even saline treatment can inhibit or facilitate lordosis when animals are divided into receptive and nonreceptive groups prior to statistical analysis[25]. The present results contrast with a previous report that ICS 205-930 (5 mg/kg) facilitates lordosis[15]; reasons for this discrepancy are not apparent. However, this is the first attempt to replicate that finding. While current data are consistent with preliminary results indicating that ICS 205-930 alone does not influence male copulatory behaviour[1], they do not support the suggestion[1] that ICS 205-930 attenuates morphine-induced inhibition of male sexual behaviour. In contrast to previous reports of blockade of morphine-evoked effects by 5-HT_3 antagonists[11–13] the current ineffectiveness of these compounds indicates that 5-HT_3 antagonists attenuate some, but not all, effects induced by morphine.

In comparison to the contributions made by 5-HT_1 and 5-HT_2 receptors, 5-HT_3 receptors appear to play a negligible role in regulating copulatory performance. This may be attributable to fundamental structural and

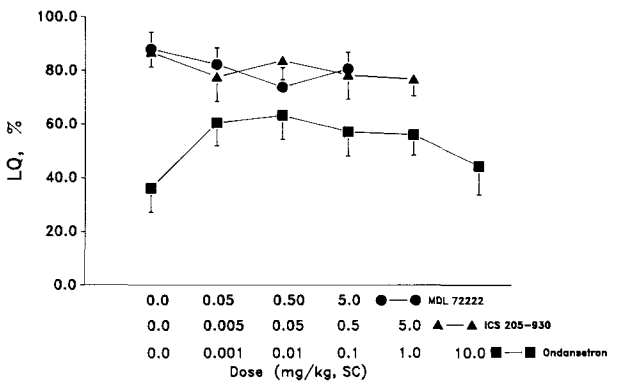

Figure 1. Effects of subcutaneously (s.c.) administered 5-HT_3 antagonists on lordosis quotient (LQ) in hormone-primed female rats. Note: variances in baseline LQs are due to different hormonal treatments as detailed in text. No significant effects were observed.

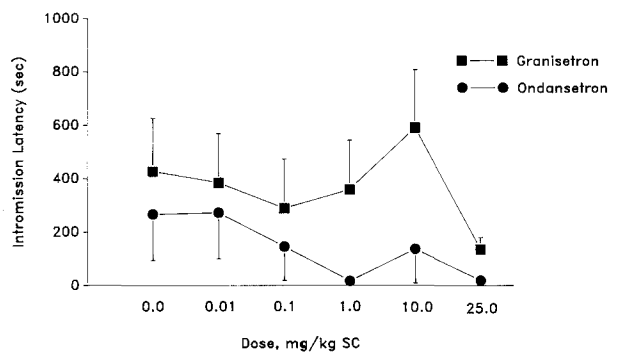

Figure 2. Effects of subcutaneously (s.c.) administered 5-HT_3 antagonists on intromission latency in male rats. No significant effects were observed.

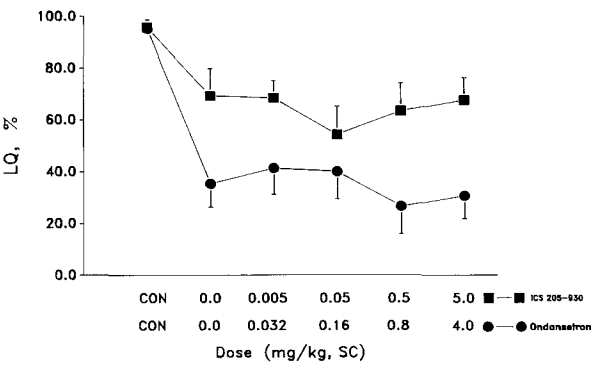

Figure 3. Effects of subcutaneously (s.c.) administered 5-HT_3 antagonists on lordosis quotient (LQ) in hormone-primed female rats. Control animals (CON) received vehicular injections only. All other animals received 2.0 mg/kg morphine s.c. Morphine significantly inhibited lordosis; this effect was not attenuated by the 5-HT_3 antagonists.

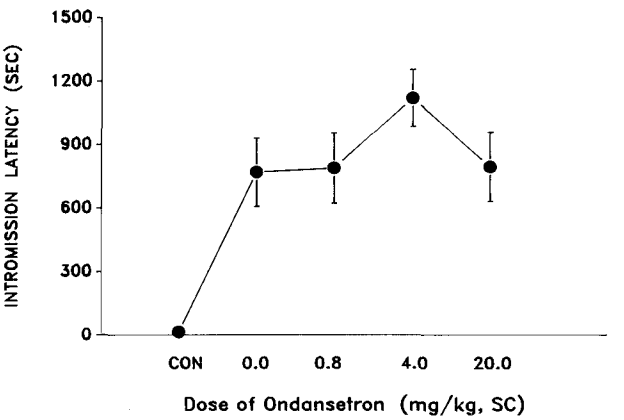

Figure 4. Effects of a subcutaneously (s.c.) administered 5-HT_3 antagonists on intromission latency (IL) in male rats. Control animals (CON) received vehicular injections only. All other animals received 1.5 mg/kg morphine s.c. Morphine significantly increased IL; ondansetron did not reverse this effect.

functional differences between 5-HT_3 and all other 5-HT receptors. Unlike other 5-HT receptor subtypes which activate GTP-binding proteins and which mediate slow, modulatory responses via second-messenger signaling pathways[26], 5-HT_3 receptors are ligand-gated, membrane ion channels which produce rapid depolarizing responses when activated[27,28]. Interestingly, it appears that 5-HT_3 receptors may be involved in mediation of the urethro-genital (UG) reflex, a reflex which consists of clonic contractions and is similar, in certain respects, to sexual climax[29]. Specifically, in female rats, the ability of peripheral 5-HT to lower the amount of urethral pressure necessary to elicit the UG reflex is blocked by the selective 5-HT_3 antagonist ICS 205-930 methiodide[29]. Although it remains to be determined whether 5-HT_3 receptors play a similar role in males, the failure of 5-HT_3 antagonists to affect ejaculation latency suggests that they do not.

Finally, as evidence suggests that receptors for which 5-HT_3 antagonists show affinity may be comprised of distinct molecular forms[22,30], it is possible that different species of 5-HT_3 receptors could have opposing effects on sexual behaviour. Activation of heterogenous 5-HT_3 sites could therefore result in a negligible net effect on sexual behaviour. Conclusive evaluation of the effects of 5-HT_3-like activity on copulatory function must await the development of more selective drugs, particularly selective 5-HT_3 agonists.

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