Examination of the 5-HT$_{2C}$ receptor agonists lorcaserin and Ro 60-0175 against nicotine discrimination and dependence in the rat

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Introduction

Selective agonists targeted to the 5-HT$_{2C}$ receptor have attracted interest as novel therapies for obesity and for the treatment of substance abuse disorders, particularly smoking cessation. We have previously reported that the 5-HT$_{2C}$ agonists Ro 60-0175 (Grottick et al, 2001) and lorcaserin (Higgins et al, 2011 in press) reliably reduce nicotine self-administration and reinstatement. The present studies were designed to expand on this potential by evaluating these drugs against a nicotine discriminative stimulus and against withdrawal signs evident following abrupt cessation of chronic nicotine exposure (Malin and Goyarzu, 2009) (see also Quarta et al, 2007; Zaniewska et al, 2007, 2010). In addition the highly selective 5-HT$_{2C}$ agonist CP-809101 (Siuciak et al, 2007) was included in some of these studies. Comparison to effects on food intake are also presented. A preliminary study to evaluate whether rats can be trained to discriminate a CP-809101 cue from saline is also presented.

Summary profile of Ro 60-0175, lorcaserin and CP-809101 across 5-HT$_2$ receptors

	h5-HT$_{2C}$	h5-HT$_{2A}$	h5-HT$_{2B}$	Ratio 2C/2A	Ratio 2C/2B
Lorcaserin (1)	7.9 (1.0)	6.7 (1.0)	6.0 (1.0)	16	80
Ro 60-0175 (2)	7.5 (0.84)	6.4 (0.69)	9.1 (0.79)	13	0.03
CP-809101 (3)	10.0 (0.93)	6.8 (0.67)	7.2 (0.57)	1585	630

Data summarised from (1) Thomsen et al (2008), (2) Porter et al (1999), (3) Siuciak et al (2007). Because of differences between cell line, receptor density, functional readout these data are best served for within drug comparisons across 5-HT$_2$ receptor subtype rather than between drug comparisons. Data shown are pEC50 and (efficacy relative to 5-HT). Note the exceptionally high functional selectivity for CP-809101 at 5-HT$_{2C}$ receptors.

Methods

Nicotine discrimination studies:: Male, Sprague-Dawley rats were trained to discriminate nicotine (0.3 mg/kg SC) from saline using a standard 2 lever choice procedure (FR10 schedule). After stable discrimination, substitution tests were conducted. Next, the effect of each drug to modify the dose related nicotine (0.03 – 0.3 mg/kg SC) generalisation was examined. In a separate cohort, rats were trained to discriminate CP-809101 (1 mg/kg SC) from saline.

Nicotine withdrawal studies: Male, Wistar rats were implanted s.c. with osmotic minipumps containing either saline or nicotine (bitartrate salt) primed to deliver drug at either 6mg/kg/day or 9mg/kg/day for 9 days. Blood levels of nicotine attained by the 9mg/kg/day regimen are considered equivalent to the range associated with heavy smokers (see Malin & Goyarzu, 2009). A precipitated withdrawal model was used by injecting rats with either mecamylamine (1mg/kg SC) or saline (control) before each test procedure. Through the use of place conditioning, locomotor activity and overt behavioural measures, an attempt was made to measure both somatic and affective withdrawal signs 7-9 days post implant (see results).

Feeding tests: Male, Sprague-Dawley rats were trained to either consume a sweetened wet mash (palatability-induced feeding) or 45mg food pellets under a progressive ratio schedule of reinforcement. After stable levels of intake, the effect of each drug on intake was determined using a within subjects design. In the palatability-induced feeding test, a behavioural measure of satiety sequence was included.

Results

A. Effect of lorcaserin, Ro 60-0175 and CP-809101 in a nicotine drug discrimination assay

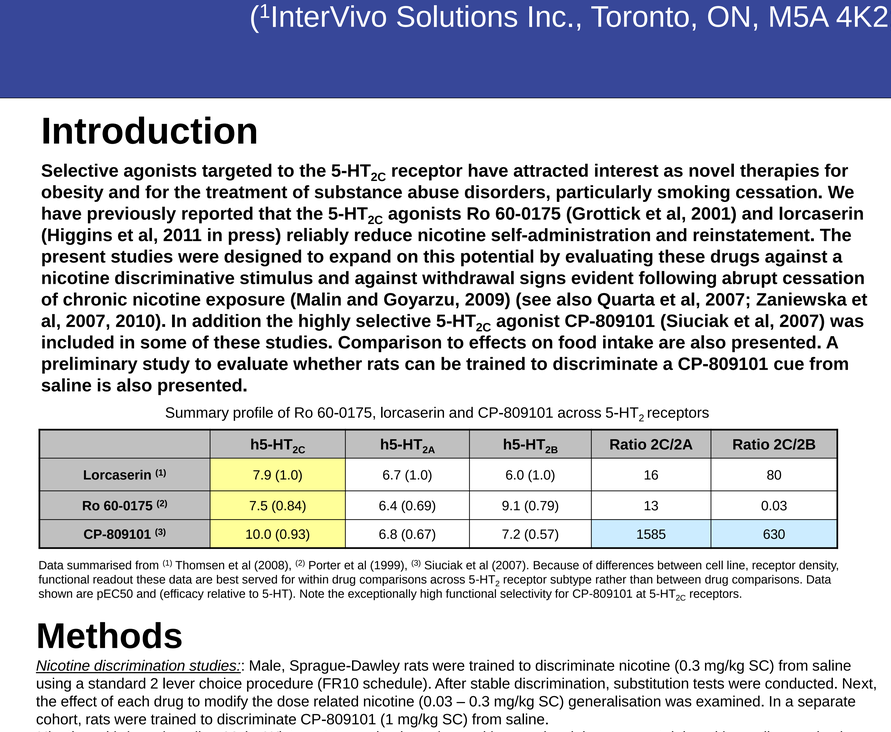

Figure 1. Failure of Lorcaserin (0.3-3 mg/kg SC), Ro 60-0175 (0.3-2 mg/kg SC), CP-809101 (0.3-6 mg/kg SC) to generalise to a nicotine (0.3 mg/kg SC) cue. Across this dose range each drug reduced response rate. Fixed dose combination of each 5-HT$_{2C}$ agonist with nicotine (0.03-0.3 mg/kg SC) antagonised the discriminative stimulus property of nicotine. In this study varenicline (0.1-1 mg/kg SC, 30min ptt.) produced only weak partial generalisation, yet the 0.3 mg/kg dose reliably attenuated a nicotine cue.

Results (cont.)

B. Characterisation of a nicotine precipitated withdrawal: effect of lorcaserin and Ro 60-0175 against somatic withdrawal signs.

B.1 Body weight change during nicotine exposure and somatic signs following a precipitated nicotine withdrawal

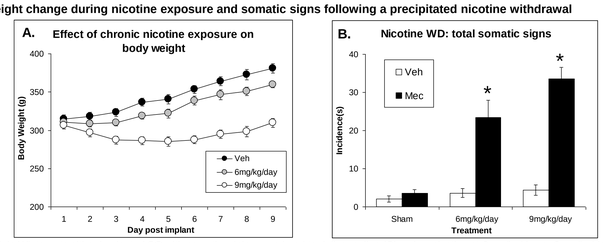

Figure 2. **(A)** Male Wistar rats either implanted SC with osmotic minipump primed to deliver saline (Sham) or nicotine at 6 mg/kg/day or 9 mg/kg/day. Some change in weight gain noted, particulary in the 9 mg/kg/day group. **(B)** On day 9, withdrawal precipitated by acute injection of mecamylamine (1 mg/kg SC) or vehicle control. Somatic withdrawal signs (e.g chewing, ptosis, wet dog shakes, twitches) measured over the subsequent 30min period – these were only evident in rats treated with the nicotine + mecamylamine combination. * P<0.05 vs. respective vehicle treated group.

B. 2 Place conditioning and locomotor activity to a precipitated nicotine withdrawal

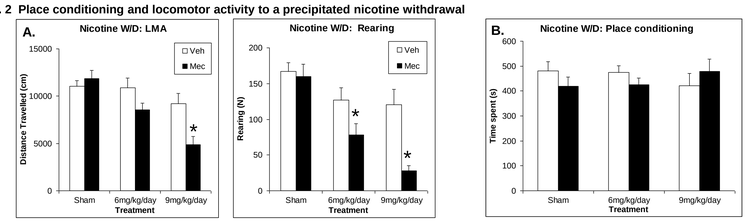

Figure 3. Effect of (□) vehicle (SC), or (■) mecamylamine (1 mg/kg SC) in Wistar rats implanted with osmotic minipump primed to deliver saline (Sham) or nicotine at 6 or 9 mg/kg/day. Locomotor activity studies conducted on day 7 post implant. In the place conditioning experiments, conditioning conducted on day 7 and 8, with preference testing on day 9. Despite clear somatic signs (see B.1) and **(A)** robust hypolocomotion we failed to identify **(B)** place aversion using a 1 trial unbiased protocol.

B. 3 Effect of lorcaserin and Ro 60-0175 against somotic signs following a precipitated nicotine withdrawal

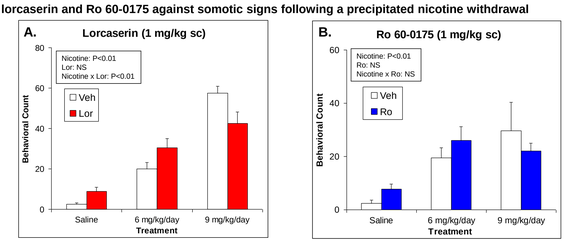

Figure 4. Effect of **(A)** lorcaserin (1mg/kg SC; 30min ptt.), **(B)** Ro 60-0175 (1mg/kg SC; 30min ptt.) against a mecamylamine (1 mg/kg) precipitated withdrawal in Wistar rats. Test conducted 9 days post minipump implantation. Neither treatment significantly affected overall incidence of withdrawal signs in each nicotine group.

B. 4 Are somatic signs of nicotine withdrawal reflective of malaise?

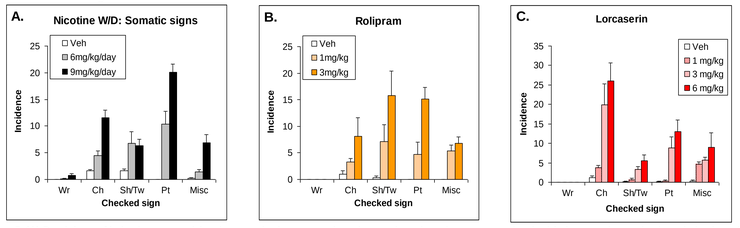

Figure 5. **(A)** Breakdown of behaviours comprising the somatic signs score show that ptosis and to a lesser extent chewing/shakes, are the predominant behaviours in our experiments. **(B)** Many of these signs are shared by treatments that induce nausea/emesis in vomiting species, e.g. rolipram (1-3 mg/kg IP recorded over 1h). This suggests that a significant proportion of somatic nicotine withdrawal signs reflect malaise. **(C)** Interestingly, lorcaserin produced similar signs at high doses (3-6mg/kg SC).

Results (cont.)

C. Characterisation of lorcaserin, Ro 60-0175 and CP-809101 in two feeding tests

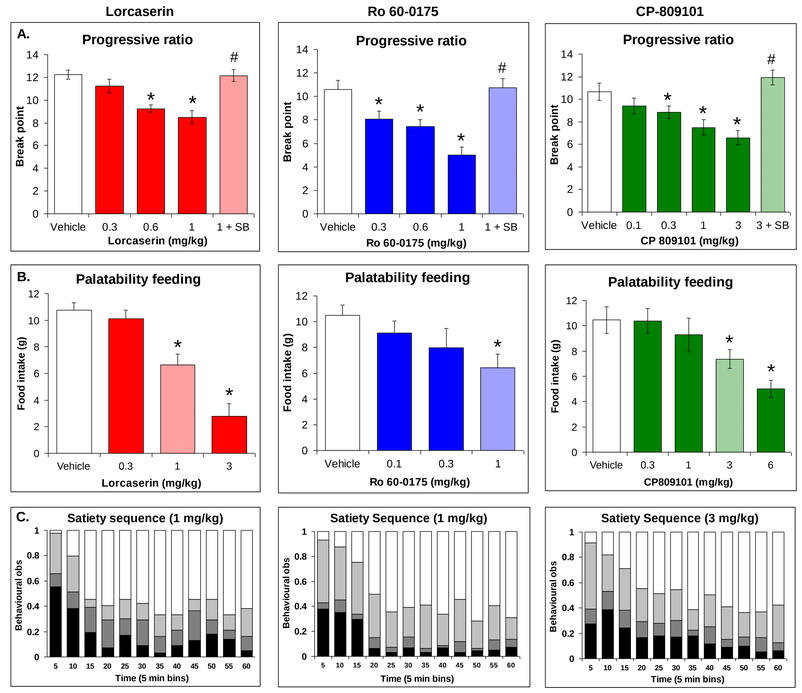

Figure 6. **(A)** Effect of Lorcaserin (0.1-1 mg/kg SC), Ro 60-0175 (0.1-1 mg/kg SC) and CP-809101 (0.1-3 mg/kg SC) on responding for food under a progressive ratio schedule of reinforcement. Reductions in break point were seen with each drug in an SB-242084 (0.5 mg/kg IP) reversible manner. N=12 rats per treatment. **(B)** Effect of lorcaserin (0.3-3 mg/kg SC), Ro 60-0175 (0.1-1 mg/kg SC) and CP-809101 (0.3-6 mg/kg SC) on consumption of a palatable sweetened mash over a 1h test period. **(C)** During the test, the behavioural sequence was also recorded. The lower graphs show the satiety sequence for each drug at the dose corresponding to the hatched histobar (i.e 1 mg/kg lorcaserin and Ro 60-0175, 3 mg/kg CP-809101). At these doses the behavioural satiety sequence was similar to control sequence. At 3mg/kg SC lorcaserin clearly disrupted the satiety sequence, which may be related to the expression of malaise related behaviours at 3-6 mg/kg SC doses (see Figure 5C). * P<0.05 vs. vehicle, # P<0.05 vs. respective 5-HT$_{2C}$ agonist alone.

D. Preliminary studies in rats trained to discriminate CP-809101: a 5-HT$_{2C}$ selective cue?

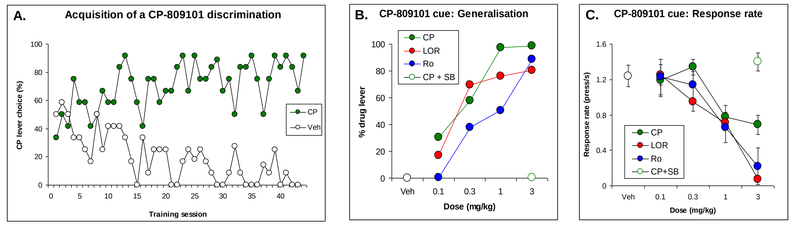

Figure 7. **(A)** Male Sprague-Dawley rats were trained to discriminate CP-809101 (1 mg/kg SC) from saline. Stable discrimination was acquired by the majority of animals by approximately 80 sessions. Response rate was significantly decreased at this training dose of CP-809101 although some tolerance developed over the course of training. **(B)** Generalisation curve for CP-809101 (0.1-3 mg/kg SC), Lorcaserin (0.1-3 mg/kg SC) and Ro 60-0175 (0.1-3 mg/kg SC) – each drug showed a dose related generalisation to the CP-809101 stimulus. The generalisation to a 3 mg/kg CP809101 dose was blocked by pretreatment with SB-242084 (0.5 mg/kg IP). **(C)** Across this same dose range response rate was also reduced in a dose related fashion by each drug. N=6-10 rats per dose. * P<0.05 vs. vehicle control.

Summary and conclusions

1. **The 5-HT$_{2C}$ receptor agonists lorcaserin, Ro 60-0175 and CP-809101 did not generalise to a nicotine cue at pharmacologically relevant doses. However each did reliably attenuate a nicotine cue, a feature similar to the nicotine partial agonist varenicline (see also Quarta et al, 2007; Zaniewska et al, 2007).**
2. **In rats chronically exposed to nicotine, acute administration of mecamylamine produced somatic behavioural signs such as ptosis, wet dog shakes, chewing and a decrease in spontaneous motor activity. At this same regimen, we were unable to observe a robust place conditioning to nicotine W/D.**
3. **Pretreatment with either lorcaserin or Ro 60-0175 (both 1 mg/kg sc) failed to attenuate the somatic withdrawal signs to a mecamylamine precipitated W/D.**
4. **Many of the W/D signs observed following nicotine W/D are similar to unconditioned behaviours observed following emetogic stimulii e.g. rolipram. This may suggest the somatic signs are reflective of a malaise, and 5-HT$_{2C}$ agonists may not modify this aspect of nicotine dependence – indeed high doses of lorcaserin produced similar signs. Interestingly Zaniewska et al (2010) recently reported that Ro 60-0175 may attenuate certain affective nicotine W/D signs.**
5. **Inhibitory effects of 5-HT$_{2C}$ agonists against a nicotine cue overlap with doses effective in feeding tests highlighting bioequivalence against food and nicotine motivated behaviour. In addition to previously reported effects of lorcaserin and Ro 60-0175 against the stimulant and reinforcing property of nicotine and in a model of relapse – these experiments support the consideration of 5-HT$_{2C}$ agonists, such as lorcaserin, as treatments to support smoking cessation.**

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