A STUDY OF THE SELECTIVE 5-HT$_{2C}$ AGONISTS LORCASERIN AND CP809101 ON SIDE-EFFECT PROFILE, CNS PENETRATION AND FUNCTIONAL SELECTIVITY

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Introduction

Clinical experience with the 5-HT$_{2C}$ receptor agonist lorcaserin (LOR) has identified efficacy in the treatment of obesity and smoking cessation, with a primary dose-limiting side-effect of nausea and headache (Chan et al, 2012). We previously reported that the highly selective 5-HT$_{2C}$ agonist CP809101 (CP) may induce fewer signs of malaise/nausea in rats, suggesting potential for 5-HT$_{2C}$ agonists with improved side-effect profiles (Higgins et al, 2013). We have adopted three lines of enquiry to further investigate. Firstly, we examined LOR and CP in the conditioned taste reactivity model, a validated rodent test to study nausea (Parker, 2014). Secondly, we compared plasma:CSF levels of LOR and CP at doses and timepoints relevant to the in-vivo studies. Thirdly, we compared the signaling profiles of LOR and CP at h5-HT$_{2C}$ receptors to look at evidence for functional selectivity differences between these two compounds (Berg et al, 1998).

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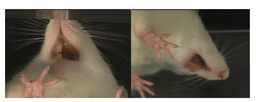
2. Chan EW, He Y, Chui CS, Wong AY, Lau WC, Wong IC (2012). Obesity Rev. 14: 383-392.
3. Higgins GA, et al (2013). Psychopharmacology 226: 475-490.
4. Higgins GA & Fletcher PJ (2015) ACS Chemical Neurosci. 6: 1071-1088.
4. Parker LA (2014) Eur J Pharmacol. 722: 122-33.

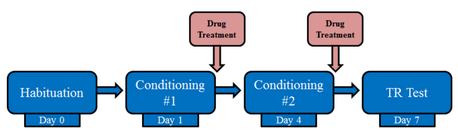
Methods

Spontaneous behaviour measurement: Male, Sprague-Dawley rats served as test subjects, Rats were singly housed and allowed to acclimatize in Perspex observation chambers for approximately 30 min before drug or vehicle treatment. Over the next 60 min each rat was observed by a trained observer for the occurrence of specific behaviours which were recorded each minute following a particular treatment. The behaviours were based on categories identified from studying rolipram: (A) chewing, i.e. orofacial movements with no apparent purpose, (B) shakes, including myoclonic jerks, paw shaking, twitches, (C) ptosis, i.e eye closure, and (D) miscellaneous behaviours which included flat body posture. With the exception of ptosis, all behaviours were scored as bout frequency, i.e treated as separate behavioural events when separated by more than 10 s. Because ptosis was often continuously displayed by affected animals, it was recorded as a separate event each minute that behaviour was displayed. To distinguish flat body posture from general behavioural inactivity, this was only scored over the first 15 min when behaviour was most obvious. In the first experiment, lorcaserin (1-6 mg/kg SC) was examined according to behavioural categories identified in experiment 1. Next, the interaction between lorcaserin (3 mg/kg SC) and SB-242084 (0.5 mg/kg IP) was examined. In a final experiment the behavioural profile of CP-809101 (3-12 mg/kg SC) treated rats was studied. Each experiment was conducted in test naïve rats according to a between subjects design (n=6 rats per group) with the observer unaware of pretreatment identity.

Taste reactivity test: Nine week old male Sprague-Dawley rats were used in both experiments (source: Charles River, St. Constant, Quebec, Canada). Animals weighed approximately 200-300g at the beginning of the study and were singly housed in polycarbonate caging. Standard laboratory chow and fresh water was provided ad libitum. The taste reactivity (TR) test involves a surgical implantation of an intraoral cannula into male, Sprague-Dawley rats. After recovery, animals are exposed to test chamber for a 5 minute habituation session (fresh water infusion). Conditioning sessions involve saccharin infusion for 3 minutes, animals receive their treatment immediately after session. On test day animals receive saccharin infusion for 3 minutes and their behaviour is monitored. Two conditioning sessions were conducted, and on Day 7 the taste reactivity to intra-oral saccharin was recorded by videocamera and subsequently measured. The gaping measure is a specific orofacial posture ("disgust reaction"; see photo below) adopted by rats on presentation of an aversive stimulus. Rats in each experiment underwent identical surgical and experimental procedures and drug vehicle was 5% tween in saline.
In Expt 1, the effect of multiple doses of lorcaserin (1, 3, 6 mg/kg SC) were tested. Lithium chloride (127 mg/kg IP) was also included for comparative purpose as a known emetogen.
In Expt 2, the effect of multiple doses of CP-809101 (3, 6, 12 mg/kg SC) were tested. Lorcaserin (6 mg/kg SC) was included as a positive control.
In Expt 3, the effect of SB-242084 (0.5 mg/kg IP) was examined against lorcaserin 6 mg/kg SC. The selected dose for SB-242084 is based on previous studies.



Gaping reaction



Conditioned taste aversion: A one-bottle CTA test was conducted after the taste reactivity test. Following a 16h water deprivation, the rats were given access to saccharin solution. The intake of saccharin was measured at 0.5h, 2h and 6h post access.

Plasma:CSF collection: Under sugical anaesthesia, male SD rats were implanted with intracerebral catheters to enable sampling of CSF. On the day following surgery, rats were treated with either LOR (0.3, 1, 3, 6 mg/kg SC) or CP-809101 (1, 3, 6, 12 mg/kg SC). 20-50uL samples of CSF and plasma (saphenous vein bleed) were collected at 0.5, 1, 2, 4, 6 and 24h timepoints. Samples were stored at -80°C before bioanalysis using LC-MS.

Functional selectivity studies: CHO cells stably expressing h5-HT2C (non-edited) receptors were used for these experiments with 5-HT as the reference agonist. The amount of [3H]-AA released or [3H]-IP accumulated was determined after 10 min of agonist incubation using our established protocols (Berg et al., 1998). ERK phosphorlyation was measured after 2.5 min incubations (see C insert) using the Surefire phospho-ERK assay Kit from PerkinElmer (Clarke et al., Br J Pharmacol, 170:532-545, 2013). Data shown in graphs were normalized to 5-HT maximal responses in each experiment and represent the mean ± SEM of 4 independent experiments. Individual CRC data were fitted by non-linear regression to determine EC50 and Emax parameters which are provided as mean ± SEM, n=4.

Results

A. Characterisation of lorcaserin and CP-809101 on spontaneous behaviour

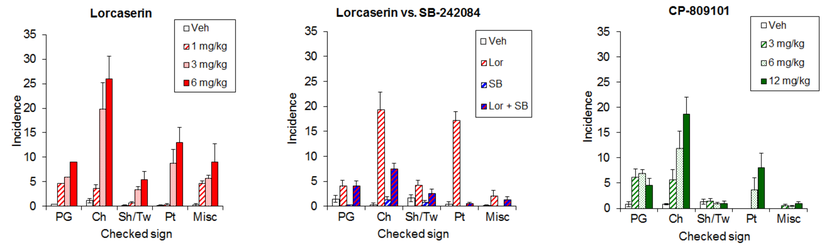


Figure 1. At relatively high doses, both lorcaserin (3-6 mg/kg SC) and CP-809101 (6-12 mg/kg SC) produced similar checked signs of vacuous chewing, ptosis, penile grooming and salivation. The effects of lorcaserin were blocked by SB-242084 (0.5 mg/kg IP). Miscellaneous (Misc) items scored in the lorcaserin study included flat body posture, chin rubbing and back muscle contracture. PG = penile grooming, Sh/Tw = shakes/twitches, Ch = vacuous chewing, Pt = ptosis.

B. Characterisation of lorcaserin and CP-809101 in the taste reactivity model

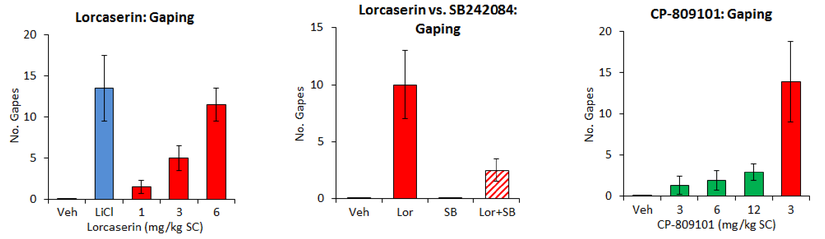
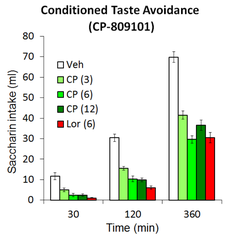


Figure 2. In separate experiments, the effect of lorcaserin, and CP-809101 were investigated in the taste reactivity model.

(A) Lorcaserin produced a dose-related increase in the number of gapes. At the 6 mg/kg dose, the effect size was equivalent to LiCl, a known emetogen.
(B) The effect of lorcaserin (6 mg/kg) on gaping was blocked by pretreatment with SB-242084.
(C) CP-809101 (3-12 mg/kg) also induced gaping although the effect size was significantly less than lorcaserin (6 mg/kg).

In a conditioned taste avoidance study, both CP-809101 and lorcaserin produce a robust CTA. In this instance, the effect size of both CP-809101 and lorcaserin is similar.



C. Measurement of blood and CSF levels of lorcaserin and CP-809101

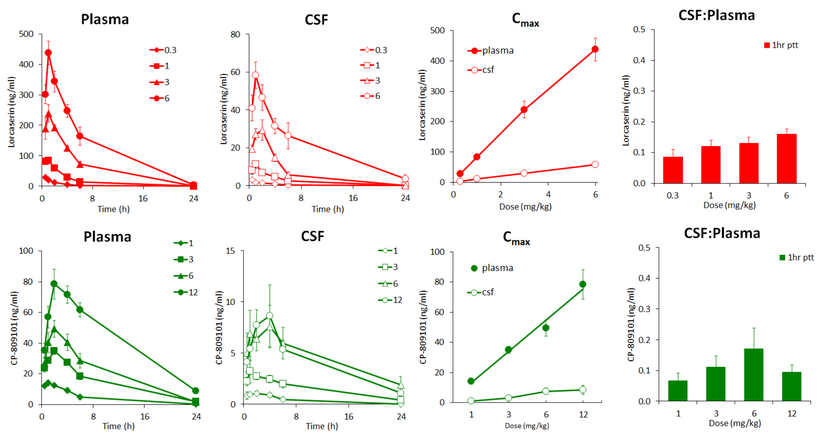
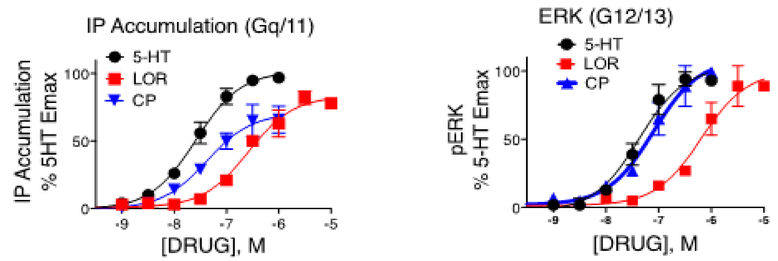


Figure 3. Increasing doses of Lorcaserin (0.3 – 6 mg/kg SC) resulted in a dose-related increase in drug concentration in both plasma and CSF compartments. Plasma:CSF ratio was similar (0.12-0.15) over the 1-6 mg/kg doses at a 1h timepoint corresponding to behavioural measures. Plasma levels of lorcaserin at 0.3 – 1 mg/kg SC were in the therapeutic dose range (i.e 20 – 80 ng/ml). In contrast, plasma levels of CP-809101 were significantly less that of lorcaserin, plasma levels at 1 mg/kg were < 20ng/ml. While CSF levels increased in dose related fashion between 1 – 6 mg/kg, there was no further increase at 12 mg/kg, thus plasma:CSF ratio declined. Since nausea is a centrally mediated effect, this lack of dose proportional CSF exposure may explain the limited side-effect profile of CP-809101.

Results

D. Functional selectivity



		IP	AA	ERK
5-HT	pEC$_{50}$ (nM)	7.34 ± 0.06 (45 nM)	7.09± 0.26 (80nM)	7.37 ± 0.21 (35 nM)
	E$_{max}$ (% above basal)	488 ± 43	306 ± 48	279 ± 73
Lorcaserin (LOR)	pEC$_{50}$ (nM)	6.44 ± 0.09 (370 nM)	6.06 ± 0.11 (870 nM)	6.01 ± 0.22 (970 M)
	E$_{max}$ (% above basal)	414 ± 17	216 ± 54*	315 ± 51
CP809101 (CP)	pEC$_{50}$ (nM)	7.28 ± 0.09 (50nM)	7.11 ± 0.23 (80 nM)	7.08 ± 0.26 (83 nM)
	E$_{max}$ (% above basal)	325 ± 18*	242 ± 31*	240 ± 28

Figure 4. Concentration response curves for lorcaserin (LOR) and CP809101 (CP) for (A) inositol phosphate (IP) accumulation (Gq/11 protein-mediated PLC activity); (B) arachidonic acid (AA) release (non-G protein mediated activation of PLA2; or (C) phosphorylation of Extracellular Signal Regulated kinase (pERK) (G12/13-protein-mediated activation of ERK, see Werry et al., J Neurochem, 93:1603-15, 2005). Data shown in graphs were normalized to 5-HT maximal responses in each experiment and represent the mean ± SEM of 4 independent experiments. (Table) Individual CRC data were fitted by non-linear regression to determine EC50 and Emax parameters which are provided as mean ± SEM, n=4. Curve fit parameters were evaluated for significant differences with One-Way ANOVA followed by Tukey's post hoc. * P < 0.05 vs 5-HT Note: For each agonist, EC50 values did not differ between signaling pathways.

Summary and conclusions

1. **Both lorcaserin (LOR) and CP-809101 (CP) are selective 5-HT$_{2C}$ receptor agonists with reliable effects on food and drug-motivated behaviours (e.g. Higgins & Fletcher, 2015). Typical dose range for these effects are 0.1 – 1 mg/kg SC.**
2. **Nausea and vomiting represent some of the most common dose limiting side effects for CNS acting drugs. Although rodents do not vomit, experimental evidence suggest they do experience nausea and respond to emetogens by a conditioned disgust reaction, i.e. gaping (Parker, 2014). Also, emotogens such as LiCl and rolipram can induce unconditioned behaviours, e.g. chewing, flat body posture (FBP).**
3. **At supra-therapeutic doses (i.e >1 mg/kg) both LOR and CP induce unconditioned behaviours such as chewing, ptosis, FBP (Higgins et al, 2013). However the magnitude of these changes are greatest for LOR suggesting this drug may produce more nausea compared to CP.**
4. **Studies using the conditioned gaping model were consistent with this view. Although both LOR and CP induced gaping, the magnitude of change was significantly greater for LOR suggesting this drug produces greater nausea compared to CP. LOR effect on gaping was reversed by SB-242084, consistent with 5-HT$_{2C}$ receptor activation.**
5. **Since nausea is a principal side-effect of LOR in the clinic (Chan et al, 2012), this may suggest that CP may represent a class of 5-HT$_{2C}$ agonist with better tolerability in humans. Explanations for this difference may relate to differences in intracellular signalling post 5-HT$_{2C}$ receptor activation, i.e functional selectivity, (see Berg et al, 1998) or differences in the CNS penetration of both drugs.**
6. **Studies conducted in CHO cells stably expressing h5-HT$_{2C}$ recetors failed to identify any potency difference between LOR and CP on IP3, ERK or AA signalling pathways. Thus no evidence for functional selectivity at the 5-HT$_{2C}$ receptor could be found between these drugs.**
7. **In contrast, while the plasma and CSF levels of LOR increase in a dose proportional manner, (plasma:CSF ratio 0.1-0.2), the CSF levels of CP appear to plateau above 6 mg/kg resulting in lack of dose proportionality.**
8. **Consequently the apparent improved tolerability of CP-809101 may be a feature of limited CNS penetration, or a substrate for efflux proteins present on the BBB, such as PgP**
9. **The present studies highlight the value to measuring plasma and CSF levels of drug in the interpretation of apparent behavioural differences between drugs of similar class.**