A STUDY OF THE SELECTIVE 5-HT$_{2C}$ AGONISTS LORCASERIN AND CP809101 ON MEASURES OF IMPULSIVITY AND FOOD REINSTATEMENT IN THE RAT

[1,4]G.A. Higgins, [1]L.B. Silenieks, [3]E. B. Altherr, [2]A. Patrick, [2]C. MacMillan, [1]I.A.M. DeLannoy, [4,5]P.J. Fletcher, [3]W.E. Pratt.

([1]InterVivo Solutions Inc., [2]Vivocore., 120 Carlton Street, Toronto, ON, M5A 4K2; [3]URECA Center, Wake Forest University, Winston-Salem, NC 27109; [4]U.Toronto, ON; [5]CAMH, Toronto, ON)

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Introduction

The 5-HT$_{2C}$ receptor agonist lorcaserin (LOR) has been FDA approved for obesity. Understanding the neurobiological processes by which 5-HT$_{2C}$ receptor agonists treat obesity may improve clinical outcomes by identifying patients who would benefit most from this pharmacological approach. Current evidence supports three potential mechanisms for anti-obesity effect of this drug class, (1) control of hypothalamic signaling of peripheral gustatory inputs, (2) control of rewarding/hedonic aspects of food, (3) regulation of motor impulsivity which may represent a predisposing factor in multiple addictive behaviours including obesity.

Study purpose was to examine the third line of evidence by examining LOR and the highly selective 5-HT$_{2C}$ agonist CP809101 (CP) on 3 food motivated impulsivity tasks (5-CSRTT, go-nogo and delay discounting), and on reinstatement of food motivated responding. Doses of each drug were directly compared to doses necessary to affect feeding induced by hunger/metabolic demand – a 22h food deprivation model. We also evaluated plasma:CSF levels of LOR at doses and timepoints relevant to the in-vivo studies for comparison to clinical exposures. Atomoxetine (ATX) was included for comparison in some tests.

Methods

Male Long Evans (LE) or Sprague-Dawley (SD) rats were used for all studies which were conducted at InterVivo Solutions, with the exception of the food reinstatement studies which were conducted at URECA Center, Wake Forest University, NC.

22h Food deprivation: Three groups of SD rats were trained to consume daily food intake in their home cage over a 2h period each day (range: 10:00 – 14:00h). By 14 days daily food intakes were stable, with consumption at 18-24g/day. Effect of LOR was investigated in 2 cohorts. Cohort 1 (N=12) received LOR at 0.3 - 1 mg/kg SC, Cohort 2 (N=8) received LOR at 0.3 - 3 mg/kg SC. A third cohort (N=12) received CP at 0.3 - 6 mg/kg SC. In each cohort, testing was conducted using a repeated measures design with 2-3 days between each treatment cycle. Food intake was measured at 1h and 2h.

5-choice task: Twenty-four male, LE rats were used. The 5-choice task was run according to standard well established techniques (e.g. Higgins et al (2012) Neuropsychopharmacol. 37: 1177-1191). For all subjects the stimulus duration was progressively reduced until a final duration of 0.6s was achieved (5s ITI, 2s LH). Training continued until subjects had achieved consistent performance above a threshold of 80% correct ([correct/(correct + incorrect)]*100) and <20% omissions for at least a two week period.
Based on pilot experiments and previously published work, doses of LOR (0.06 - 0.6 mg/kg SC), and CP (0.1 - 1 mg/kg SC) were selected. Doses of each drug were tested in all 24 rats using a cross-over design. Atomoxetine (ATX; 0.5 - 1 mg/kg IP) was included as a comparitor at the completion of the LOR and CP studies.

Go-NoGo successive discrimination task: Twenty male LE rats were trained to the task based on the methods described by Harrison et al (Psychopharmacol. 217: 455-473; 2011). Briefly, the rats were initially trained to lever press for food reward (45mg food pellet). Following acquisition of the lever press response, rats were trained to a symmetrically reinforced GO/NOGO (lever press/no lever press) conditional visual discrimination task in response to a stimulus light cue (fast 0.1s/5Hz or slow 0.75s/0.5Hz) to receive food reward, i.e to GO or NOGO. A typical session consisted of 40 GO and 40 NOGO trials presented in a random sequence, lasting 10s each (approximate session duration 20 min). The primary measure is the animals' efficiency in terms of correct responses/total responses made during the GO and NOGO period. False alarms reflect lever press responses made during a NOGO trial, and failure to correctly respond during a GO trial is recorded as an error. Response latencies are also recorded. Both LOR (0.1 – 0.6 mg/kg SC) and ATX (0.1 – 2 mg/kg IP) were tested in all animals.

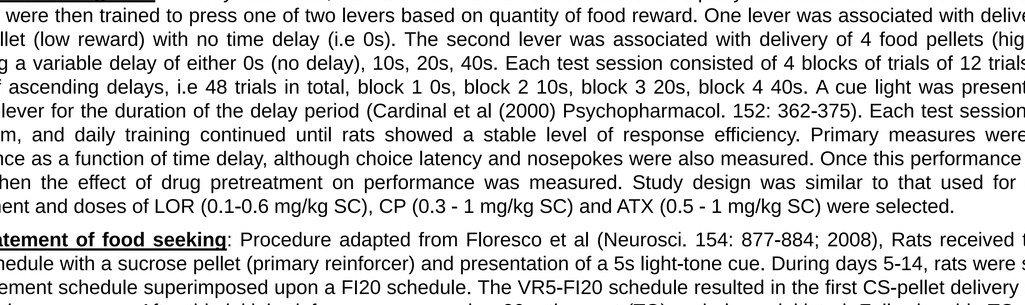
Delay discounting task: Twenty-four male, LE rats were used. Once trained to equally associate both levers with food reward the animals were then trained to press one of two levers based on quantity of food reward. One lever was associated with delivery of a single food pellet (low reward) with no time delay (i.e 0s). The second lever was associated with delivery of 4 food pellets (high reward) but following a variable delay of either 0s (no delay), 10s, 20s, 40s. Each test session consisted of 4 blocks of trials of 12 trials per block, in order of ascending delays, i.e 48 trials in total, block 1 0s, block 2 10s, block 3 20s, block 4 40s. A cue light was presented above the chosen lever for the duration of the delay period (Cardinal et al (2000) Psychopharmacol. 152: 362-375). Each test session was 48 trials maximum, and daily training continued until rats showed a stable level of response efficiency. Primary measures were high reward preference as a function of time delay, although choice latency and nosepokes were also measured. Once this performance measure was stable then the effect of drug pretreatment on performance was measured. Study design was similar to that used for the 5-CSRTT experiment and doses of LOR (0.1-0.6 mg/kg SC), CP (0.3 - 1 mg/kg SC) and ATX (0.5 - 1 mg/kg SC) were selected.

Reinstatement of food seeking: Procedure adapted from Floresco et al (Neurosci. 154: 877-884; 2008), Rats received training on an FR1 schedule with a sucrose pellet (primary reinforcer) and presentation of a 5s light-tone cue. During days 5-14, rats were shifted to VR5 reinforcement schedule superimposed upon a FI20 schedule. The VR5-FI20 schedule resulted in the first CS-pellet delivery on average of 5 active lever presses. After this initial reinforcer was earned, a 20 s time-out (TO) period was initiated. Following this TO, the VR5-FI20 schedule was repeated until the 20 min session was complete. Following 10 days of VR5-FI20 training, rats underwent daily 20 min extinction sessions in which active and inactive lever presses resulted in no programmed consequences. Rats considered to reach extinction (ready for reinstatement testing) when they responded <10% compared to the last 3 days of VR5-FI20 training.
Reinstatement testing. One day after each rat achieved extinction criterion, they were subjected to 2x20min reinstatement sessions separated by 48h. Reinstatement sessions consisted of the renewed presentation of the light-tone cue following the first lever press on the previously active lever. Further responding resulted in cue presentation on a VR-5 schedule. There was no sugar pellet delivery on reinstatement days, to specifically assess the cue-evoked incentive to lever press. Four individual groups of rats were tested. Across the two test days, rats received in counterbalanced order, a saline injection and a single drug treatment (LOR 0.1, 0.3, or 0.6 mg/kg SC; or CP 1 mg/kg, SC.). The second reinstatement session for each rat was separated by a 48h interval.

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 LOR (0.3 – 6 mg/kg SC) and 20-50uL samples of CSF and plasma (saphenous vein bleed) collected at 0.5, 1, 2, 4, 6 and 24h timepoints. Samples were stored at -80°C before bioanalysis using LC-MS.

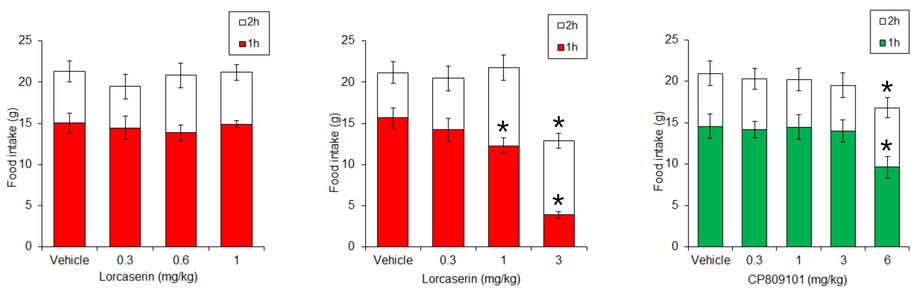
Results

Measurement of plasma and CSF levels of Lorcaserin (0.3 – 6 mg/kg SC):



Lorcaserin (0.3 – 6 mg/kg SC) produced a dose related increase in drug concentration in the plasma and csf compartments. Plasma:csf ratio was in the range 0.2 across all doses. Therapeutic dose range is approx. 40-50 ng/ml (e.g. Aronne et al, 2015; Postgrad. Med. 126, 7-18) attained by lorcaserin doses 0.3 -0.6 mg/kg SC 1-2h post dosing. At doses of 3 mg/kg and above (>200 ng/ml plasma), clear signs of malaise become evident.

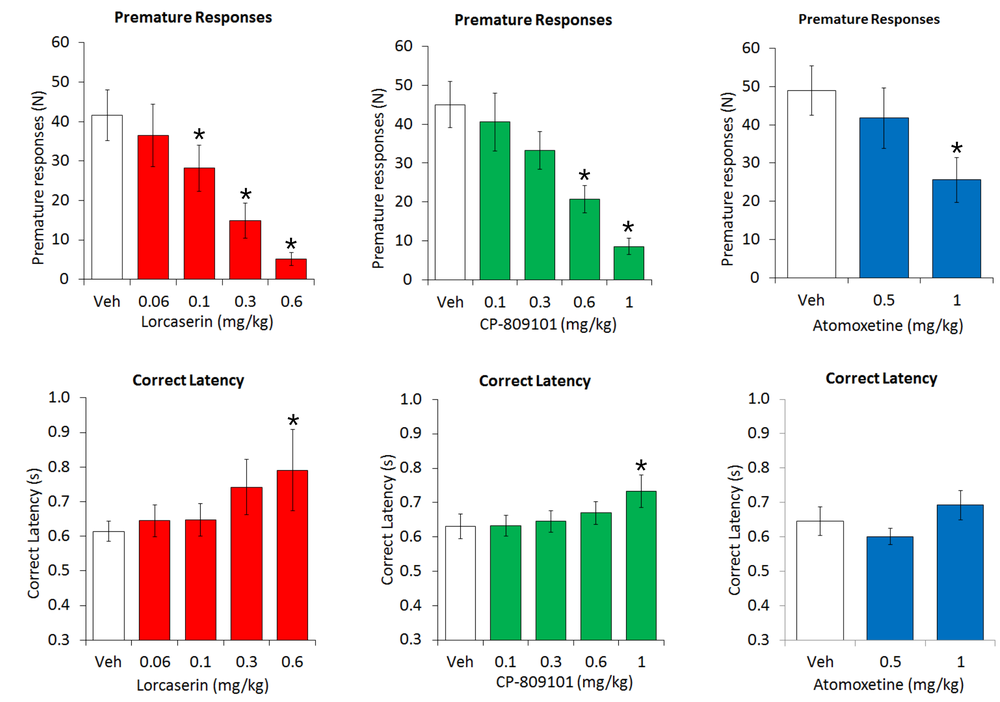
Deprivation-induced feeding:



Lorcaserin was tested in 2 cohorts. In Cohort #1 (N=12) Lorcaserin (0.3-1 mg/kg SC) had no effect on deprivation-induced feeding. In Cohort #2 (N=8) Lorcaserin (0.3-3 mg/kg SC) did reduce feeding at the 3 mg/kg dose. A significant decrease in intake was also recorded at 1 mg/kg SC (1h only). In cohort #3 (N=12) CP-809101 (0.3 – 6 mg/kg SC) only reduced intake at 6 mg/kg dose.

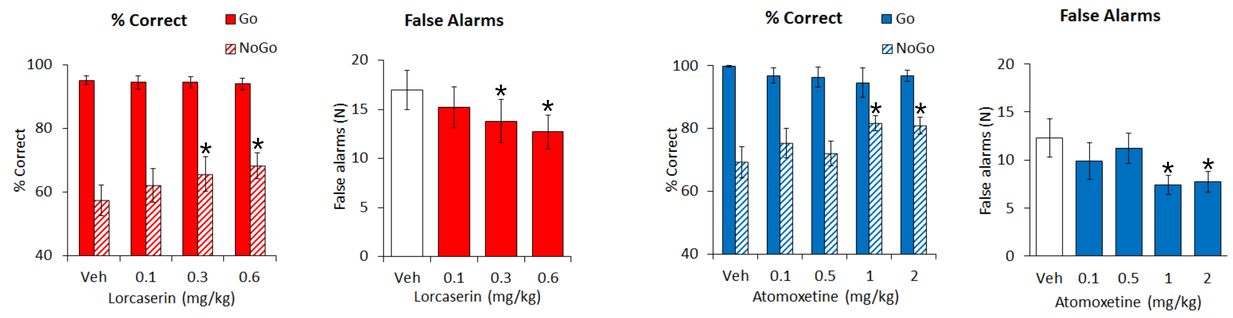
Results (cont.)

5-Choice Serial reaction time task (impulsive action):



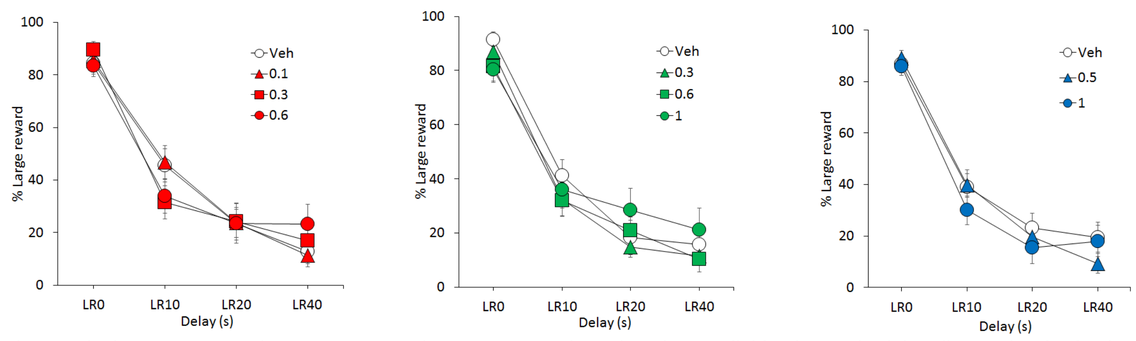
Lorcaserin (0.1 – 0.3 mg/kg SC) reduced premature responding without significantly affecting speed of responding. At 0.6 mg/kg LOR slowed response speed and increased omissions. Accuracy was unaffected across all doses. CP-809101 (0.3 – 1 mg/kg SC) showed a similar response pattern suggesting with careful dose titration 5-HT$_{2C}$ agonists can selectively reduce this measure of impulsive action. ATX (0.5 - 1 mg/kg IP) shared a similar profile.

Go-NoGo task (impulsive action):



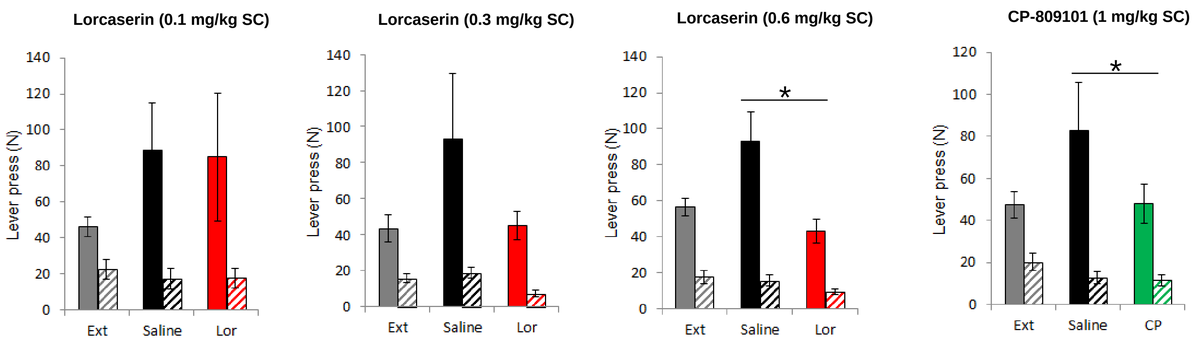
Rats readily acquired the GO task phase faster than the NOGO, and consistently performed the GO at a higher level of accuracy (GO: >95%; NOGO 60-70% correct). Pretreatment with lorcaserin (0.1 - 0.6 mg/kg SC) and ATX (0.1 - 2 mg/kg IP) reduced false alarms and consequently improved accuracy on the NOGO test phase. Performance on GO test phase was unaffected. Overall % correct improved by LOR and ATX.

Delay discounting task (impulsive choice):



Neither lorcaserin (F3,57 = 0.1, NS) nor CP-809101 (F3,57 = 1.5, NS) produced any reliable change in delay discounting for food reward. This null effect was in sharp contrast to the effect of both 5-HT$_{2C}$ agonists in tests of impulsive action. ATX was similarly ineffective.

Reinstatement of food seeking:



Lorcaserin (0.3 – 0.6 mg/kg SC) reduced cue-induced reinstatement of food seeking behaviour. CP-809101 (1 mg/kg SC) was similarly effective. Thus both 5-HT$_{2C}$ agonists are active in this model of dietary relapse.

Summary and conclusions

1. **LOR and CP reduced both premature responding in 5-CSRTT and false alarms (NoGo responses) in the Go-NoGo task. Effects were seen at relatively low doses of each drug having no effect on deprivation-induced feeding (i.e feeding motivated by hunger) and were similar in magnitude and robustness to ATX.**
2. **Neither LOR nor CP produced any reliable effect on delay discounting for food reward at equivalent doses to those effective in the 5-CSRTT and Go-NoGo tasks.**
3. **Thus both 5-HT$_{2C}$ agonists appear to modulate behaviours characterised as impulsive action but not choice – apparently similar to 5,7 DHT lesion (Winstanley et al, 2004). ATX showed a similar profile.**
4. **Given associations between impulsive action and propensity to reinstate drug seeking behaviour, we examined both LOR and CP in a reinstatement model of food seeking.**
5. **Both LOR and CP attenuated cue-induced reinstatement of food seeking behaviour.**
6. **Data support proposal that 5-HT$_{2C}$ agonists promote weight loss clinically at least in part through regulation of impulsive action and reducing relapse to dietary regimens. 5-HT$_{2C}$ agonists may be effective in promoting weight loss in individuals whose obesity is linked to eating conditions associated with high impulsive trait, e.g. Binge eating disorder (Schag et al, 2013). Studies also support 5-HT$_{2C}$ agonists as treatments for substance abuse, where high impulsivity may be a predisposing factor.**
7. **Doses of LOR active in tests of impulsive action and cue reinstatement (0.3-0.6 mg/kg SC) result in plasma levels of 20-60 ng/ml which are equivalent to clinical plasma levels (44 ng/ml; Aronne et al, 2015).**