EFFECT OF LORCASERIN AND ATOMOXETINE IN TWO TESTS OF COMPULSIVE ACTION

[1]G.A. Higgins, [2]M. Brown, [2]J. St. John, [2]C. MacMillan, [2]L.B.Silenieks
([1]InterVivo Solutions Inc., Toronto, ON M5A 4K2, Canada; [2]Vivocore Inc.,Toronto, ON, Canada)

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Introduction

Although the 5-HT_{2C} receptor agonist lorcaserin (LOR; Belviq®) has been approved for obesity, there is a view that this drug may be effective in other eating disorders, such as binge eating disorder (BED), as well as other clinical conditions characterised by addiction (e.g. Higgins et al, 2016a). The selective noradrenergic reuptake inhibitor atomoxetine (ATX; Strattera®), approved for ADHD, has also been considered as a treatment for BED. Both LOR and ATX are effective in reducing behaviours related to impulsive action, which may contribute to effective treatment of BED. The present investigations were designed to examine both drugs in three test challenges of compulsive action – (1) schedule-induced polydipsia (SIP) in "High" drinkers, (2) extended (10s) ITI in perseverative (PSV) responding in the 5-choice task (5-CSRTT), and (3) dizocilpine (DZP)-induced increase in PSV responding in the 5-CSRTT. Premature responses (PREM) a measure of motor impulsivity was also measured in these 5-CSRTT experiments.

Methods

Schedule-induced polydipsia

Background: The SIP task is a model of obsessive compulsive behaviour in rodents (Moreno & Flores, 2012). Due to anthropomorphic limitations of using rodents to measure obsessive thoughts, OCD models are limited to evaluation of the compulsive and repetitive behaviors of animals. Of these, models of adjunctive behaviors offer value. Schedule-induced polydipsia (SIP) is an adjunctive model in which hungry (non-thirsty) rats exhibit exaggerated drinking behavior (polydipsia) when presented with food pellets under an uncontrollable, low reinforcement rate provided by a fixed-time schedule, i.e the drinking behaviour is a consequence of the periodic food delivery. The polydipsic response is an excessive expression of a normal behavior (water drinking), providing face validity to the model. Similar to other tasks that measure neuropsychiatric traits, the SIP model of compulsive drinking identifies individual differences between rats and also differences among rat strains.

Animals and housing: Male Wistar rats (source: Charles River, St. Constant, Quebec, Canada) served as test subjects. Rats were singly housed with water available ad-libitum. Food was restricted to approximately 20g/day. Rats of the Wistar strain tend to have a higher incidence of high intensity drinking behaviour (i.e. compulsive drinkers), relative to other strains and so have become a frequently used strain for SIP studies (Merchan et al, 2019).

Schedule-induced polydipsia (SIP): Male Wistar rats were be given access to food (45mg food pellets) under a FI60s schedule for 1h/day for 5-6 days/week in an operant test chamber. Coincident with food presentation the rats were given access to water through a water bottle (~50mL volume) placed in the test chamber. In addition to checking that all pellets are consumed, the amount of water consumed by the rat in the 1h test session was measured. Over a period of approximately 2 months (20-30 sessions), a subgroup of rats reliably consumed a significant volume of water (~20ml) during the 1h session (termed high drinkers). Once water intakes were stable over days, drug testing began in the high drinker cohort.

The effect of lorcaserin (0.1-0.6 mg/kg SC), CP809101 (0.3-1 mg/kg) and atomoxetine (0.1-1 mg/kg IP) was investigated in separate groups of high drinker rats (N=8 per group). Drug testing was conducted according to a repeated measures design with each animal receiving each treatment over repeated test sessions run 2 days/week (typically Tuesday-Friday or Weds-Saturday). On intervening days (except Sunday) the rats were run under baseline conditions to allow drug washout between cycles. Both drug testing and baseline sessions would be identical in design, (i.e. FI60s schedule, 1h duration).

Control tests of drinking and food intake: In separate cohorts of male Wistar rats, the effect of lorcaserin (0.1-0.6 mg/kg SC), CP809101 (0.3-1 mg/kg SC) and atomoxetine (0.1-1 mg/kg IP) was investigated on (1) drinking induced by 18h water deprivation over a 60min test period (no food deprivation), and (2) time to consume 60 food pellets placed in a dish in home cage (food deprivation as per SIP experiments). Drug testing was conducted according to a repeated measures design (N=8 rats per treatment).

5-choice serial reaction time task

Background: The 5-choice serial reaction time task (5-CSRTT) is widely used to measure attentional performance in rodents (e.g. Higgins and Silenieks, 2017). The basic test design involves training animals to respond to a brief visual stimulus presented unpredictably in one of five locations. Once trained to stable performance levels, the effects of experimental manipulations on response speed and choice accuracy are measured and each are related to attentional performance. The test is also widely used to examine aspects of response control with responding prior to stimulus onset, i.e. a premature response (PREM), becoming a widely used measure of motor impulsivity. Responses made after a correct response but prior to the collection of food reward are termed perseverative responses (PSV) and have been described as a measure of compulsive action since they are repetitive with some negative consequence (delay to food reward). The test is adaptable to task modification, for example, extending the ITI from 5s to 10s will reduce the frequency of stimulus presentation and increase PREM responses. The long ITI manipulation has become widely used to tax performance.

Animals and housing: Male Long Evans rats (source: Charles River, St. Constant, QE) were used in all experiments. The rats were singly housed and food restricted to ~20g food/day. Body weight of rats was ~350g at onset of testing.

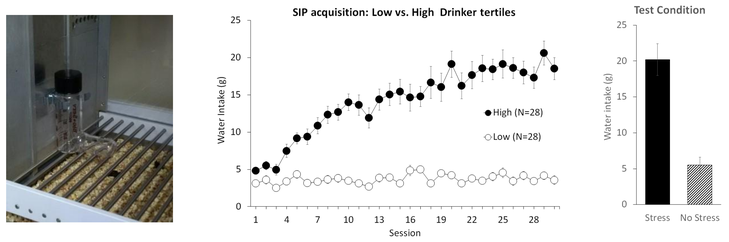
5-choice task: Rats were trained to asymptotic performance in the 5-CSRTT over a period of approximately 2 months. The rats were trained to final stimulus duration (SD) of 0.75s, 5s inter-trial interval (ITI), 5s limited hold (LH), 100 trials per session. Target performance levels under these conditions were in the range of >80% accuracy, <20% omissions. Once performance stable, drug testing and/or test challenge began. Drug testing was be conducted according to a repeated measures design with each animal receiving each treatment over repeated test sessions – test sessions run 1-2x weekly to allow drug washout and rebaseline between cycles. Animals were run 5-6 days/week under standard test conditions with experimental challenges presented on test days only. The effect of lorcaserin (0.1-0.6 mg/kg SC), CP809101 (0.3-1 mg/kg SC) and atomoxetine (0.1-1 mg/kg IP) was investigated in separate cohorts of rats (N=8-24 per group depending on experimental schedule).

LONG 10s ITI challenge: Rats tested under conditions of long ITI (i.e 100 trials, SD=0.3s, ITI=10s, LH=5s) presented 1-2x weekly. Rats were subgrouped into "Low impulsive" (LI) and "High impulsive" (HI) based on PREM responding under vehicle pretreatment (Higgins and Silenieks, 2017).

Dizocilpine challenge: NMDA antagonist dizocilpine (0.03 mg/kg SC) produces a robust increase in PREM and PSV under standard test conditions (Higgins et al, 2016b). Dizocilpine or vehicle were administered 5min pre-test session, test compound or vehicle 10-30min pre-test session.

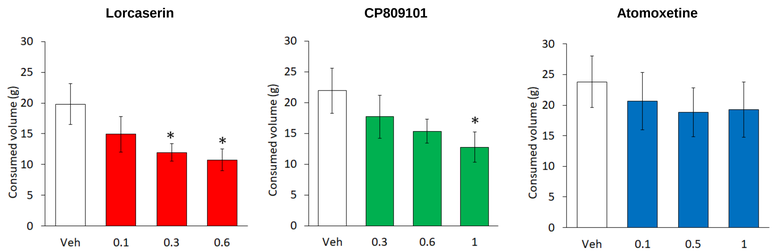
Results (Schedule-induced polydipsia)

Summary acquisition data for SIP drinkers (N=84 Wistar rats)



Operant test box fitted with a drinking tube placed on the rear wall, opposite to the food magazine. Food dispensed under a FI60s schedule over a 60min test period, i.e 60 pellets/session. Rats free to consume water throughout the test period. Total water volume consumed is the primary measure. Over 20+ training sessions, rats show individual differences in drinking despite all rats consuming the 60 food pellets. The rats may be separated based on tertiles into "High drinkers", and "Low drinkers". Drug testing is conducted in "High drinker" cohort once intakes stabilized, typically after 20 sessions. Test condition compares water intake for N=8 High drinkers under "Stress" condition, i.e regular test condition, or "No stress" condition, i.e all 60 pellets presented at start of test session.

Effect of lorcaserin, CP809101 and atomoxetine on Schedule-induced polydipsia



Effect of the selective 5-HT_{2C} receptor agonists lorcaserin (0.1-0.6 mg/kg SC), and CP809,101 (0.3-1 mg/kg SC), and the selective NA reuptake inhibitor atomoxetine on water consumption measured over a 60min test session in "High drinker" cohort. Drugs were tested according to a repeated measures design (N=8 rats per drug). Both lorcaserin and CP809101 reliably reduced water intake, atomoxetine in contrast produced an inconsistent effect. *P<0.05 vs. Vehicle control (Dunnetts test following significant ANOVA).

Effect of lorcaserin, CP809101 and atomoxetine on deprivation-induced drinking and free food intake

	Lorcaserin				CP809101				Atomoxetine			
	Veh	0.1	0.3	0.6	Veh	0.3	0.6	1	Veh	0.1	0.5	1
Dep. Drinking (g)												
30min	33.8±0.9	27.8±2.1	31.2±1.8	30.5±3.1	28.5±2.7	30.6±2.4	31.5±2.8	32.4±1.6	28.1±2.6	30.3±1.0	26.0±0.8	26.9±4.6
60min	36.3±0.9	30.7±2.4	33.4±1.5	33.3±2.6	34.2±3.3	36.0±2.8	35.1±2.6	38.1±2.5	30.3±2.6	35.0±1.8	28.5±1.4	29.7±5.2
Free feeding (s)	119±12	114±10	144±21	160±23 *	88±4	93±6	105±13	103±9	98±9	98±9	98±11	95±10

Effect of the selective 5-HT_{2C} receptor agonists lorcaserin (0.1-0.6 mg/kg SC), and CP809,101 (0.3-1 mg/kg SC), and the selective NA reuptake inhibitor atomoxetine on water consumption induced by 18h deprivation. Intake measured at 30min and 60min of test session. An additional control test was to give rats access to 60 food pellets and measure time to consume the food. *P<0.05 vs. Vehicle control (Dunnetts test following significant ANOVA) (N=8 rats per experiment).

Summary and conclusions:

1. **The 5-HT_{2C} receptor agonists lorcaserin (0.3-0.6 mg/kg) and CP809101 (1 mg/kg) reduce SIP at doses that did not disrupt free feeding or deprivation-induced water intake.**
2. **In contrast, the NA reuptake inhibitor atomoxetine (0.1-1 mg/kg) did not reliably affect SIP.**
3. **In the 5-CSRTT, extending the training ITI from 5s to 10s, produced a reliable increase in PREM and PSV responses, measures of impulsive and compulsive action respectively. PSV co-segregated with PREM responses in tertile subgroups based on PREM responses.**
4. **Lorcaserin, CP809101 and atomoxetine, each reliably decreased both PREM and PSV responses in a dose related fashion, notably in animals categorized as "High Impulsive".**
5. **The non-competitive NMDA antagonist dizocilpine (0.01-0.06 mg/kg SC) increased PREM and PSV responses in animals tested under a standard 5-CSRTT schedule.**
6. **Lorcaserin, CP809101 and atomoxetine, each reliably decreased PREM induced by dizocilpine (0.03 mg/kg). The effect of each drug against PSV responses was less clear but there were trends for each to decrease this measure.**
7. **While reliable affects of lorcaserin, CP809101 and atomoxetine were noted against indices of impulsive action, effects against indices of compulsive action were more complex and require further study. Both 5-HT_{2C} receptor agonists suppressed compulsive action as measured in the SIP paradigm and increased PSV responses produced by schedule and pharmacological manipulation. However, the effect was variable.**

References:
Higgins et al, (2016a) Psychopharmacol. 233(14): 2841-2856.
Higgins et al (2016b) Behav. Brain Res. 311: 1-14.
Higgins & Silenieks (2017) Rodent Test of Attention and Impulsivity: The 5-Choice Serial Reaction Time Task. Curr Protoc Pharmacol. 78:5.49.1-5.49.34.
Merchan et al, (2019) Prog Neuropsychopharmacol Biol Psychiatry. 93: 149-160.
Moreno & Flores (2012) SIP as a model of compulsive behavior: neuropharmacological and neuroendocrine bases. Psychopharmacol 219(2):647-659.

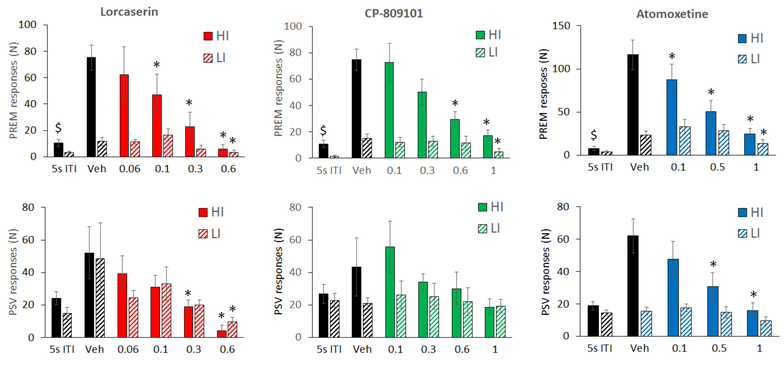
Results (5-choice task)

Effect of extended 10s ITI on PSV and PREM responding in 5-choice task (N=92 rats)

	N	ITI	% Correct	Omissions	Total PSV	% PSV	Total PREM	% PREM	Total # trial
All subjects	92	5s	90.5±0.8	10.2±1.1	19.9±1.7	21.7±2.4	7.4±0.9	7.7±1.0	96.2±1.4
	92	10s	75.2±1.3 *	17.3±1.3 *	50.1±2.7	80.6±6.8	61.9±4.5 *	81.3±6.0 *	82.2±2.6 *
"Low" impulsive (LI)	30	5s	91.4±1.5	12.7±2.4	14.3±2.1	15.3±2.4	3.7±0.8	3.7±0.8	94.0±3.4
	30	10s	79.3±2.2 *	17.0±2.3 *	18.8±2.0	27.7±2.5	22.7±1.9 *	38.9±6.4 *	74.3±5.1 *
"High" Impulsive (HI)	30	5s	90.1±1.3	7.7±1.5	27.2±3.7	30.7±6.1	12.0±2.2	12.6±2.2	98.0±2.0
	30	10s	73.1±1.8 *#	16.3±2.0 *	67.8±7.3	79.1±8.2	111.1±7.2 *#	131.2±9.2 *#	89.3±3.8 *#

Summary of performance of Long Evans rats in the 10s ITI 5-choice schedule. Metanalysis of 92 rats tested under this schedule – comparison of performance for accuracy (% correct), premature responding (total # PREM and % PREM) and perseverative responding (total # PSV and % PSV) between 5s ITI (0.75s SD) and 10s ITI (0.3s SD). Rats show a wide variation in terms of # PREM and PSV responses emitted during a 10s ITI session. These differences are reasonably consistent across repeated testing. Ranking rats according to # premature responses – low tertile group (N=30) (LI) and high tertile group (N=30) (HI). Note the co-segregation of PSV responding with PREM responses under this schedule.

Effect of lorcaserin, CP809101 and atomoxetine against extended ITI-induced increase in PREM and PSV responses



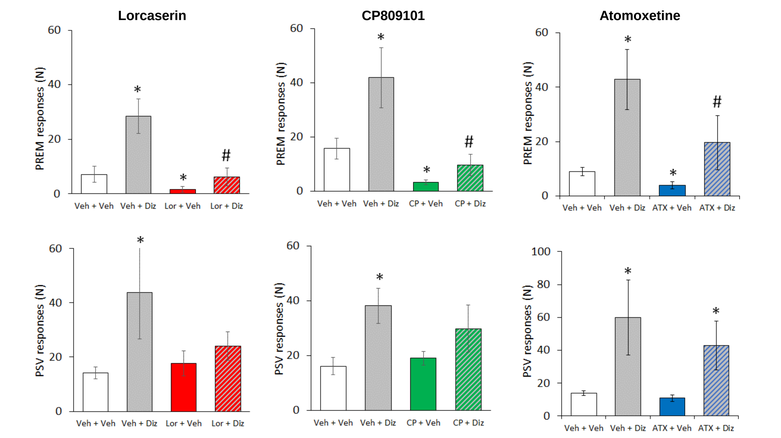
Effect of lorcaserin (0.1-0.6 mg/kg SC), CP809,101 (0.3-1 mg/kg SC), and atomoxetine on PREM and PSV responses in the 10s ITI 5-choice schedule. Drugs were tested according to a repeated measures design. All treatments significantly reduced PREM responses, notably in the HI tertile. All treatments also reduced PSV responses although in the CP809,101 experiment no overall main effect was measured. *P<0.05 vs. Vehicle control, $P<0.05 vs. LI rats on 5s ITI (Dunnetts test following significant ANOVA) (N=8 rats per tertile, 24 rats total).

Effect of dizocilpine on PSV and PREM responding in 5-choice task (N=51 rats)

	% Correct	Omissions	% Hit	Total PSV	% PSV	Total PREM	% PREM	Total # trial
Vehicle	86.4±1.3	7.8±0.9	77.8±1.7	20.7±1.6	23.3±1.8	7.8±0.9	8.8±1.0	89.8±2.2
Dizocilpine 0.01 mg/kg	87.7±1.3	4.1±0.5	83.2±1.4	33.4±3.7	42.4±6.6	17.5±3.0	22.7±4.2	87.5±2.2
Dizocilpine 0.03 mg/kg	88.9±1.4	5.6±0.8	82.9±1.6	58.7±9.7 *	124.0±32.2 *	34.5±7.2 *	76.3±20.4 *	82.4±3.4
Dizocilpine 0.06 mg/kg	81.2±2.0	29.9±4.0 *	55.3±3.8 *	52.7±8.4 *	91.3±21.2 *	24.2±6.1 *	48.8±14.1 *	85.0±3.1

Summary of performance of Long Evans rats pretreated with dizocilpine (0.01-0.06 mg/kg SC) in the 5s ITI 5-choice schedule. Analysis of 51 rats tested under this schedule. Dizocipline produced a robust increase in PREM and PSV responses at 0.03-0.06 mg/kg SC dose range. At 0.01-0.03 mg/kg a trend to increase % hit, but at 0.06 mg/kg and above attentional performance begins to decline. * P<0.05 vs. Vehicle pretreatment (Dunnett test following significant ANOVA).

Effect of lorcaserin, CP809101 and atomoxetine against dizocilpine (0.03 mg/kg)-increase in PREM and PSV responses



Effect of lorcaserin (0.6 mg/kg SC), CP809,101 (1 mg/kg SC), and atomoxetine (1 mg/kg IP) on increased PREM and PSV responses induced by dizocilpine (0.03 mg/kg; Diz). All treatments reduced PREM responses both in Vehicle and Diz pretreated rats. All treatments also showed a trend to attenuate the Diz-induced increase in PSV responses. *P<0.05 vs. Vehicle control (Dunnett test following significant ANOVA) (N=8 rats per experiment).