Modulation of Striatal Adenosinergic Function by HTL0041178, a Selective GPR52 Agonist

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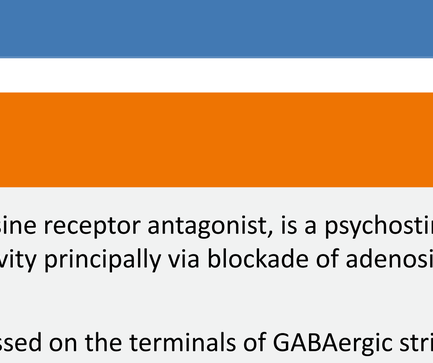
Background

- Caffeine, a non-selective adenosine receptor antagonist, is a psychostimulant which increases rodent locomotor activity principally via blockade of adenosine 2A (A_{2A}) receptors (El Yacoubi et al, 2000)
- A_{2A} receptors are densely expressed on the terminals of GABAergic striatopallidal neurons in the indirect pathway of the basal ganglia, in which dopamine D_2 receptors are co-expressed. Tonic activation of A_{2A} receptors decreases the affinity of the D_2 receptor to dopamine, while antagonism of A_{2A} facilitates dopaminergic signalling (Ferré, 2008)
- Several antipsychotic agents have been shown to block caffeine-induced hyperlocomotion (Batista et al, 2016) and indeed the adenosine hypothesis of schizophrenia posits that hyperdopaminergia may be secondary to a loss of function of the adenosine system (Lara et al, 2006)
- GPR52, a constitutively active Gαs G protein-coupled orphan receptor, is predominantly expressed in the striatum on D_2 striatopallidal neurons (Komatsu et al, 2014) and a recent spatiomolecular mapping study showed a distinct overlap of GPR52 with A_{2A} receptors in a subpopulation of striatal neurons (Märtin et al, 2019)
- The aim of the present study was to explore whether HTL0041178, a selective GPR52 agonist (Watson et al, 2021), would modulate rat hyperlocomotor activity stimulated by caffeine or the selective A_{2A} receptor antagonist istradefylline

Locomotor activity driven by A_{2A} inhibition in GPR52-hub D_2-expressing cells



Methods

Evaluation of GPR52 expression in the rat brain

- Staining of paraffin-embedded sagittal brain sections from Sprague Dawley rats was performed using a rabbit polyclonal antibody raised to GPR52 (GeneTex; GTX108123) and chromogenic DAB staining.

Locomotor studies

- After 1h habituation to the locomotor cages, male Sprague-Dawley rats (n=12 per group) were dosed with vehicle, risperidone (0.6 mg/kg, IP) or HTL0041178 (3, 10 and 30 mg/kg, PO)
- One hour later, they were dosed with vehicle/caffeine (15 mg/kg, SC) for the caffeine study, or vehicle/istradefylline (10 mg/kg, IP) for the istradefylline study, and locomotor activity was assessed for 2h
- Data are back-transformed means, adjusted for differences between treatment groups in activity during the 30 minutes prior to treatment with test compound. They were analysed using a general linear model with treatment, cohort and cage rack as factors. HTL0041178 was compared to vehicle by Williams' test
- Samples were taken at the end of the study to confirm plasma and brain concentrations of HTL0041178

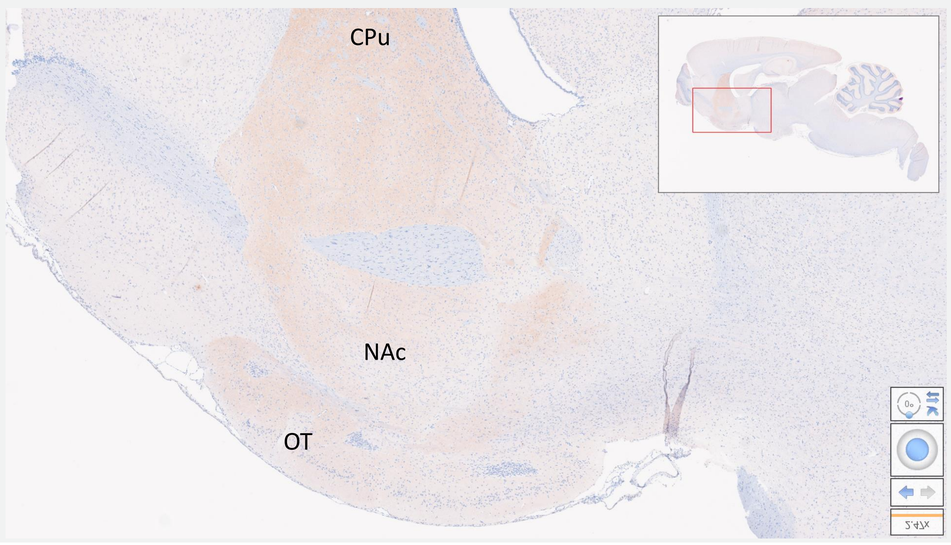
In vitro competition assay

- Striatal membrane suspensions were incubated with HTL0041178 (1 µM) for 10 minutes and then with [^{3}H]ZM241385 and either assay buffer (total binding), SCH442416 (non-specific binding), caffeine (10 concentrations 100-1E6 nM) or istradefylline (10 concentrations 0.01-1,000 nM) for 30 minutes
- Log-transformed data were analysed by two-way analysis of variance with treatment and assays as factors

Results

Evaluation of GPR52 expression in the rat brain

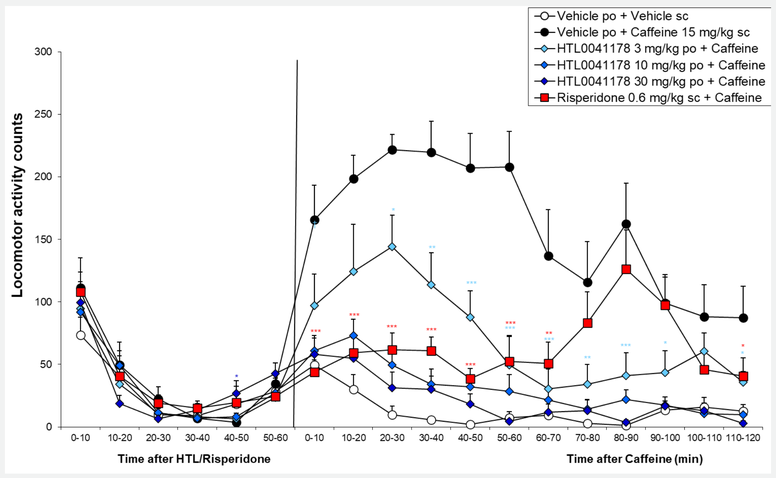
- Histochemical analysis of GPR52 expression in sagittal brain sections from Sprague Dawley rats. A single representative image is shown demonstrating expression in the striatum (including caudate putamen (CPu), nucleus accumbens (NAc) and olfactory tubercle (OT)).



Results continued

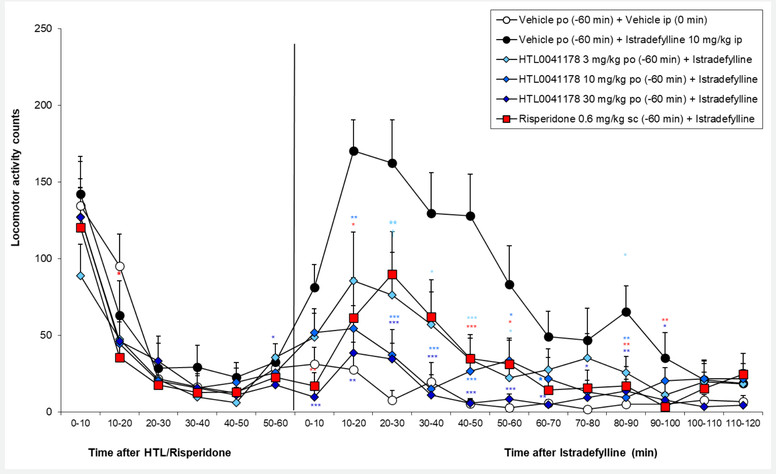
Caffeine-induced Hyperlocomotion in the Rat

- Treatment with HTL0041178 resulted in a dose-dependent reduction of caffeine-induced hyperlocomotion at all doses tested. Risperidone also significantly reduced caffeine-induced hyperlocomotion. * p<0.05, ** p<0.01, *** p<0.001 vs vehicle-treated caffeine group.



Istradefylline-induced Hyperlocomotion in the Rat

- Treatment with HTL0041178 resulted in a dose-dependent reduction of istradefylline-induced hyperlocomotor responses at all doses tested. Risperidone also significantly reduced istradefylline-induced hyperlocomotion . * p<0.05, ** p<0.01, *** p<0.001 vs vehicle-treated istradefylline group.



In Vitro Competition Assay

- The presence of HTL0041178 had no effect on the affinity of caffeine or istradefylline for the A_{2A} receptor as measured in the in vitro [^{3}H]ZM241385 binding assay

Treatment	n	Mean	SEM	%	p
SCH 442416	4	2.12	0.12		
SCH 442416 + 1 µM HTL0041178	4	1.81	0.12	85	0.192
Istradefylline	3	4.74	0.44		
Istradefylline + 1 µM HTL0041178	3	5.47	0.27	115	0.296
Caffeine	3	10666	1397		
Caffeine + 1 µM HTL0041178	3	9667	601	91	0.468

Conclusions

- The highly selective (against a panel of 47 other targets) GPR52 agonist HTL00411718 was found to modulate the behavioural response to A_{2A} receptor antagonists without directly affecting A_{2A} receptor binding
- Interestingly, Nishiyama et al (2017) demonstrated that the locomotor response to istradefylline was significantly augmented in GPR52 KO mice compared to WT mice
- Due to its localisation on D_2 neurons, GPR52 has been proposed as a target for the treatment of psychosis but its specific co-expression with the A_{2A} receptor and a potential role in the interplay between the adenosinergic and dopaminergic systems warrants further investigation.

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