A microdialysis and behavioural comparison of lisdexamfetamine and methylphenidate in freely-moving rats

Helen Rowley PhD1; David Hackett MSc2; Rajiv Kulkarni PhD1; David Heal PhD, DSc1
RenaSci Ltd, Nottingham, UK; 2Shire Pharmaceuticals Ltd, Basingstoke, UK

INTRODUCTION
Lisdexamfetamine dimesylate (LDX; Vyvanse®), is a prodrug comprising d-amphetamine (d-AMF) covalently linked to L-lysine that is metabolized by red blood cells to produce its active metabolite, d-AMF (Pennick, 2010). Methylphenidate is a pharmacologically active stimulant. Both compounds are used for the treatment of ADHD and are Schedule 2 Controlled Drugs in the USA. LDX is currently in development in Europe. Enhancement of catecholaminergic neurotransmission in the prefrontal cortex (PFC) and striatum (STR) is important for the therapeutic actions of drugs used to treat ADHD. However, supra-therapeutic doses of these drugs rapidly induce dopaminergic neurotransmission in the brain to produce euphoria, thereby placing them at serious risk of diversion, recreational abuse and dependence.

We have used the Culex Bambino (BAS, Inc) to compare the effects in freely-moving rats of therapeutic and supra-therapeutic doses of LDX or immediate-release methylphenidate on brain monoamine efflux in the PFC and STR by dual probe microdialysis and simultaneous locomotor activity measurement.

METHODS
Male Sprague Dawley rats (250-350g; Charles River, UK) were used (n=4-7). Two concentric microdialysis probes (CMA) were implanted into PFC (2mm tip; AP: +3.2 mm, L: +/-2.5 mm from bregma, V: -4 mm) and STR (4 mm tip; AP: +0.2 mm, L: +/-3 mm from bregma, V: -7.6 mm) under isoflurane/O2 anesthesia according to Paxinos & Watson (1986). Animals were allowed to recover 2-6 h in the Culex Bambino dialysis bowls with food and water available ad libitum. The probes were perfused continuously with artificial CSF at a 1.2 µl/min flow-rate. The effects of oral doses of LDX (d-AMF base = 0.5, 1.5, 4.5 mg/kg, po) and methylphenidate (3, 10, 30 mg/kg, po) on extracellular levels of noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) in PFC and STR were determined. Dialysate samples were automatically collected into refrigerated vials containing 5 µl 0.1M perchloric acid every 15 min. Locomotor activity was determined using the Raturn® system. Monoamine neurotransmitter concentrations were quantified using the Alexys HPLC-electrochemical detection system (Antec). Probe placements were verified histologically at the end of the experiment.

RESULTS
• Comparing the intermediate [therapeutic] doses of LDX and methylphenidate, both compounds increased efflux of NA and DA in the PFC (Fig 1A,B). The effects of LDX on DA and NA were slower in onset those of methylphenidate, but more consistently significant at later time-points. LDX significantly increased the efflux of NA, DA and 5-HT across all doses, whereas the profile of methylphenidate changed with dose and the drug had no enhancing effect on 5-HT (Fig 1C).
• Comparing the intermediate doses of LDX and methylphenidate in the STR, LDX increased the efflux of DA and sporadically also 5-HT, whereas the only significant effect of methylphenidate was to increase DA efflux at a single time-point (Fig 2A,B). LDX dose-dependently increased both striatal DA and 5-HT efflux (Fig 2C). Although methylphenidate enhanced striatal DA efflux, its effects were smaller than LDX and at two doses methylphenidate actually decreased extracellular 5-HT (Fig 2C).
• The intermediate doses of LDX and methylphenidate caused only transient significant increases locomotor activity at one or two time points (Fig 3A). Sustained and significant increases in locomotor activity were only seen after administration of the highest [supratherapeutic] doses of LDX and methylphenidate (Fig 3B). When the correlation between the extracellular concentration DA and the magnitude of locomotor activity was determined for the highest doses of LDX and methylphenidate, it was evident that the slope of the relationship was much steeper for methylphenidate than for LDX (Fig 3C).

CONCLUSIONS
• The pharmacological profile of LDX was markedly different from methylphenidate by virtue of its action to increase the extracellular levels of DA, NA and 5-HT in PFC and DA and 5-HT in STR. By contrast, the therapeutically equivalent doses of methylphenidate had an exclusively catecholaminergic action.
• The pharmacological profile of LDX on central monoaminergic function did not vary with dose, whereas that of methylphenidate did.
• At therapeutically relevant doses, LDX produced larger and more sustained increases in the extracellular concentrations of monoamines in the PFC and STR than methylphenidate.
• LDX produced much larger increases in striatal DA efflux than methylphenidate with a much lower propensity to induce locomotor activation.
• 5-HT has an attenuating influence on dopaminergic neurotransmission in the STR and this may account for LDX’s less stimulant profile compared with methylphenidate.
• Together, these data predict that LDX may provide a greater separation between efficacy and stimulant adverse events in the clinical situation than methylphenidate.

ACKNOWLEDGEMENTS
This research was performed by RenaSci Ltd with funding from Shire Pharmaceutical Development Ltd.