Characterisation of the catecholaminergic profiles of methylphenidate and its enantiomers in an animal model of ADHD by in vivo microdialysis.

RenaSci Consultancy Ltd, Nottingham, NG1 1GF, UK.

INTRODUCTION
Attention deficit hyperactivity disorder (ADHD) is characterised by four primary symptoms, i.e. impulsiveness, inattentiveness, distractibility and overactivity. These abnormalities are also present in the behavioural repertoire of the spontaneously hypertensive rat (SHR), and consequently, this strain has been proposed to be an excellent model of human ADHD.\(^1,^2,^3\)

\(\text{dl-}\text{threo-Methylphenidate (}\text{dl-MPH}\text{, which is an established treatment for attention deficit hyperactivity disorder (ADHD), exists as 2 enantiomers, i.e. }\text{d-}\text{ and }\text{l-MPH}.\text{ This study has determined the contribution of their effects to the actions of the parent racemate (}\text{dl-MPH}\text{) on extracellular levels of dopamine (DA) in the striatum and noradrenaline (NA) in the frontal cortex of freely-moving SHRs using dual-probe microdialysis.}

MATERIALS AND METHODS
Male, SHRs (250–290 g; Charles River, UK) were anaesthetised using isoflurane (5% to induce, 2% to maintain) in an \(\text{O}_2/\text{N}_2\text{O}\) mixture (1 litre/min each).

A concentric dialysis probe (CMA, UK, 2 mm tip) was stereotaxically implanted into the prefrontal cortex (coordinates: AP: +3.2 mm; L: +/-2.5 mm relative to bregma; V: -4.0 mm relative to the skull surface) and striatum (AP: +0.2 mm; L: +/-3.0 mm; V: -7.8 mm). Coordinates are according to Paxinos and Watson.\(^4\)

Rats underwent a recovery period of at least 16 h during which time food and water were available ad libitum and probes were continuously perfused with an artificial cerebrospinal fluid (Harvard Apparatus, UK) at a flow rate of 1.2 µl/min.

Four basal samples (15 min interval) were collected prior to intraperitoneal (ip) administration of drug or saline. Sample collection continued for 4 h post-drug administration into Eppendorf vials which contained 5.0 µl of 0.1 M perchloric acid to prevent oxidation.

Samples were stored at -80°C until analysis for DA and NA by HPLC with electrochemical detection.

Values are mean ± SEM (\(n = 8-13\)) and statistical comparisons were made between drug- and saline-treated groups by one-way ANCOVA with Williams’ test for multiple comparisons.

RESULTS

Figures 1 and 2 show that the parent racemate, \(\text{dl-MPH}\), produced rapid dose-related elevations in the efflux of striatal DA and cortical NA with maximum increases ~60min after dosing. The effects on extraneuronal concentrations of DA were approximately twice as great as on NA, i.e. at 20mg/kg 729 ± 232% (\(P<0.001\)) versus 469 ± 69% (\(P<0.001\)) compared to baseline. However the effects on NA were longer lasting being significantly higher than baseline at the end of the experiment, whereas DA had returned to control values at this time.

Figures 3 and 4 show the pharmacodynamics of the effects of \(\text{d-MPH}\) were similar to those of the racemate with peak increases in DA and NA of 1042 ± 117% (\(P<0.001\)) and 449 ± 51% (\(P<0.001\)), respectively. By contrast, the \(\text{l-enantiomer was much less potent producing only a 200 ± 24% (\(P<0.01\)) increase in DA efflux with no significant effect on NA.}

CONCLUSIONS

This is the first study to have systematically defined the catecholamine profile of \(\text{d-MPH}\) and its enantiomers in vivo.

In the SHR model of ADHD, \(\text{dl-MPH}\) produced powerful effects on DA and NA efflux in vivo. As the dose was increased, its dopaminergic effect became more pronounced relative to its noradrenergic actions.

The comparison of the enantiomers of MPH revealed that \(\text{d-MPH}\) markedly increased extraneuronal concentrations of DA in the striatum and NA in the prefrontal cortex. By comparison, the effect of \(\text{l-MPH}\) was much very weaker and was restricted to a significant increase of DA alone.

The magnitude of effects of \(\text{d-MPH}\) on DA and NA efflux compared with those of \(\text{l-MPH}\) demonstrate that the former is the predominant contributor to the pharmacological actions of the parent racemate.

REFERENCES

ACKNOWLEDGEMENTS
This experimental study was sponsored by Shire Pharmaceuticals Ltd.

Presented at Neuroscience, Atlanta, 2006.
**Partnering pharma, biotech and virtual companies to develop the next generation of drugs for obesity, metabolic and CNS disorders**

RenaSci is a contract provider of consultancy and experimental services. Our specialists have proven track-records in discovering novel drugs to treat obesity, diabetes and a wide range of CNS disorders combined with “molecule to market” experience. RenaSci provides effective solutions via consultancy and strategic advice, experimental projects or an integrated blend of both. RenaSci has no ‘in house’ drug discovery to produce conflicts of interest. If required, we can provide documentation to regulatory standards. Confidentiality is guaranteed.

**Experimental and Consultancy Services**

**Experimental Services**

**Metabolic diseases**
- Obesity
- Diabetes
- Metabolic Syndrome

**Neurology**
- Cognition
- Parkinson’s disease

**Psychiatry**
- Psychosis
- Abuse liability

**Consultancy**

- Preclinical Discovery and Development
- Clinical Development Planning
- Strategic and Marketing

[www.renasci.co.uk](http://www.renasci.co.uk)

or contact our Head of Business Development:

Rob Jones (MBA, PhD)
E-mail: rob.jones@renasci.co.uk
Telephone: +44 (0) 115 912 4262