A microdialysis and behavioural investigation of modafinil in freely-moving rats

H. L. Rowley[1], R. S. Kulkarni[1], D. Hackett[2], D. J. Heal[1]
[1] RenaSci Ltd, BioCity, Nottingham, NG1 1GF, UK. [2] Shire Pharmaceutical Development Ltd, Basingstoke, RG24 8EP, UK.

INTRODUCTION

Modafinil (Provigil®) is an unusual stimulant that is widely used to treat narcolepsy in USA and Europe. It has an enigmatic mechanism of action (see reviews by Minzenberg & Carter, 2008; Heal et al, 2012). Modafinil has stimulant-like effects in humans and animals, but its pharmacology is different from the catecholaminergic stimulants *d*-amfetamine and methylphenidate. Although modafinil has low micromolar affinity for DAT (Zolkowska et al., 2009; Madras et al., 2006) and no affinity for NET or SERT (Madras et al, 2006; Minzenberg & Carter, 2008), positron emission tomography (PET) experiments have paradoxically revealed that therapeutic doses of modafinil occupy a substantial proportion of striatal DAT sites in the brains of humans (Volkow et al, 2009). Modafinil also decreased [^{11}C]raclopride binding (Volkow et al, 2009), indicating occupancy of DAT sites resulted in increased synaptic concentrations of dopamine.

With stimulant drugs, pharmacological mechanisms mediating their therapeutic actions are also responsible for their side-effects (see Heal et al, 2012). Consequently, a fine balance must be maintained between maximising therapeutic efficacy without inducing unacceptable levels of adverse events. The aim of this study was, therefore, to compare the neurochemical and behavioural effects of various doses of modafinil to obtain a greater insight into the balance between efficacy and adverse events. This objective was achieved by combining dual-probe microdialysis sampling of noradrenaline, dopamine and 5-HT in the brains of freely-moving rats with simultaneous locomotor activity measurements. The brain regions selected were the prefrontal cortex (PFC) and striatum because both areas are important for the therapeutic actions of stimulant drugs and the latter mediates many of the psychomotor side-effects of the stimulants.

METHODS

Experiments were carried out in male Sprague-Dawley rats (250-350g; Charles River). Two concentric microdialysis probes were stereotaxically implanted under isoflurane anesthesia into A) the PFC (2 mm tip, coordinates: AP: +3.2 mm; L: +/- 2.5 mm relative to bregma; V: -4.0 mm relative to the skull surface) and B) the striatum (4 mm tip, coordinates: AP: +0.2 mm; L: +/- 3.0 mm; V: -7.8 mm) according to the atlas of Paxinos and Watson (1986). Following surgery, rats were individually housed in the Culex Bambino (BASi, West Lafayette IN) dialysis bowls and allowed to recover for at least 16 hr with *ad libitum* food and water. Probes were continuously perfused with aCSF (sodium 150 mM; potassium 3 mM; magnesium 0.8 mM; calcium 1.4 mM; phosphate 1.0 mM; chloride 155 mM) at a flow rate = 1.2 µl/min. On the day of the experiment, dialysate samples were collected from freely-moving rats into vials containing 5.0 µl of 0.1 M perchloric acid at 15 min intervals from 60 min before until 5 hr after drug administration (4 basal samples, 20 post-drug samples). Measurement of noradrenaline, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT and 5-hydroxyindolacetic acid (5-HIAA) in dialysis samples was by reverse-phase, ion-pair HPLC coupled with electrochemical detection using an ALEXYS monoamine analyzer (Antec, Leyden).

Modafinil free-base (Tocris Bioscience, Bristol) was suspended in 1% methylcellulose in deionized water and dosed by the clinically relevant oral route of administration.

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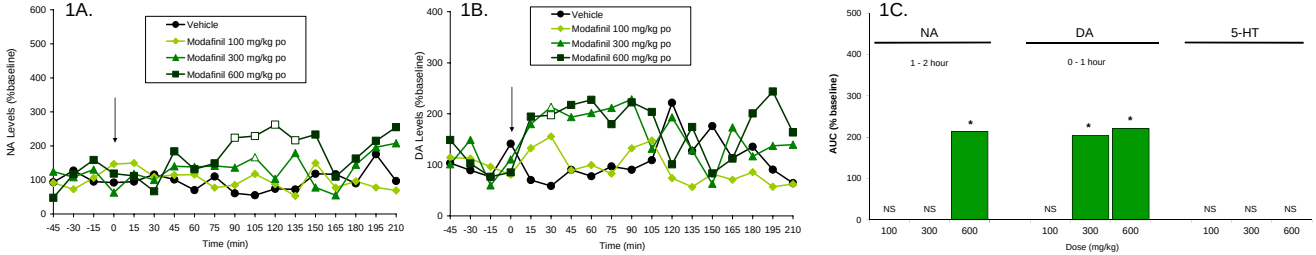
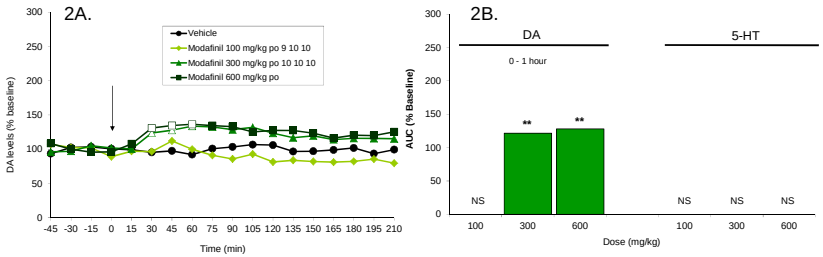
Fig 1. Effect of modafinil on extracellular monoamine levels in the PFC



Data are means ± SEM; n = 5-8. Means were back-transformed and adjusted for differences between treatment groups at baseline. SEMs were calculated from the residuals of the statistical model. For clarity, the SEMs are not shown on the figures. SEM values as percentages of the mean were: Vehicle = 14-48; Modafinil = 16-54 (100 mg/kg, po), 26-65 (300 mg/kg), 27-70 (600 mg/kg). Time of administration is indicated by the vertical arrows. Data analysed by ANCOVA with each dose of drug compared to vehicle controls by Williams' test. Significant differences denoted by open symbols. p<0.05.

Data are the AUC (% baseline) (n=5-6) in the extracellular concentrations of NA, DA and 5-HT that were statistically significant at the time point indicated. Results were analysed by ANCOVA with each dose of modafinil compared to the vehicle-treated control group by Williams' test. Significant differences are denoted by *p<0.05.

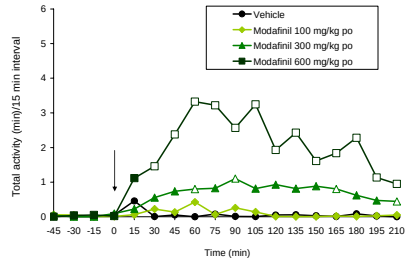
Fig 2. Effect of modafinil on extracellular monoamine levels in the striatum



Data are means ± SEM; n = 5-8. Means were back-transformed and adjusted for differences between treatment groups at baseline. SEMs were calculated from the residuals of the statistical model. For clarity, the SEMs are not shown on the figures. SEM values as percentages of the mean were: Vehicle = 8-12; Modafinil = 10-33 (100 mg/kg, po), 10-18 (300 mg/kg), 10-15 (600 mg/kg). Time of administration is indicated by the vertical arrows. Data analysed by ANCOVA with each dose of drug compared to vehicle controls by Williams' test. Significant differences are denoted by the open symbols. p<0.05.

Data are the AUC (% baseline) (n=5-8) in the extracellular concentrations of DA and 5-HT that were statistically significant at the time point indicated. Results were analysed by ANCOVA with each dose of modafinil compared to the vehicle-treated control group by Williams' test. Significant differences are denoted by **p<0.01

Fig 3. Effect of modafinil on locomotor activity



Data are means ± SEM; n = 5-8. Means were back-transformed and adjusted for differences between treatment groups at baseline. SEMs were calculated from the residuals of the statistical model. For clarity, the SEMs are not shown on the figures. SEM values as percentages of the mean were: Vehicle = 0-0.8; Modafinil = 0-1.1 (100 mg/kg, po), 0-9.0 (300 mg/kg), 0-15 (600 mg/kg). Time of administration is indicated by the vertical arrows. Data was analysed by ANCOVA with each dose of drug compared to the vehicle-treated control group by Williams' test. Significant differences are denoted by the open symbols. p<0.05.

RESULTS

- In the PFC, modafinil (300 and 600 mg/kg po) significantly increased the extracellular concentrations of noradrenaline (Figure 1A) and dopamine (Figure 1B). These effects were most pronounced in the first 2.0 hr after administration and supported by AUC analysis (Figure 1C). This stimulant was without effect at the lowest dose of 100 mg/kg. Modafinil (100 - 600 mg/kg po) did not alter 5-HT efflux in the PFC (Figure 1C).
- In the striatum, modafinil (300 and 600 mg/kg po) increased dopamine efflux (Figure 2A,B). The increases were rapid in onset and lasted for approximately 1 hr and were approximately equal in magnitude at both doses (Figure 2A,B). Modafinil was without effect at the lowest dose of 100 mg/kg (Figures 2A, B).
- Modafinil (100 - 600 mg/kg po) did not alter striatal 5-HT efflux (Figure 2B).
- Modafinil (100 - 600 mg/kg po) produced moderate dose-dependent increases in the locomotor activity of the rats (Figure 3). However, the locomotor activity evoked by modafinil was not significantly correlated with the increase in extracellular dopamine in the striatum (r^2 = 0.024 at 300 mg/kg; r^2 = 0.002 at 600 mg/kg).

CONCLUSIONS

- The results revealed that the lowest dose of 100 mg/kg po of modafinil was at the threshold of pharmacological effect. Higher doses of modafinil increased the extracellular concentrations of noradrenaline and dopamine in PFC and dopamine in the striatum.
- Although the magnitude of modafinil's effect on extraneuronal catecholamine concentrations in the PFC is similar to that observed with lisdexamfetamine or methylphenidate (Rowley et al, 2012), the increase in striatal dopamine efflux was much smaller.
- Modafinil dose-dependently increased locomotor activity. The lack of correlation between striatal dopamine efflux and motor activity suggests other neurotransmitter(s) have a role in mediating its behavioural effects.
- The current findings that modafinil has a much greater ability to enhance catecholaminergic function in the PFC than dopaminergic neurotransmission in the striatum, and in addition, that another as yet unknown neurochemical mediator may be the primary driver of modafinil's stimulant actions help to explain why modafinil has an unusual pharmacological profile.