**EX VIVO OCCUPANCY AS A TECHNIQUE TO ASSESS DRUG ACTIONS AT MULTIPLE TARGETS**

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**INTRODUCTION**

There is currently a resurgence of interest in drugs acting on multiple molecular targets, but for compounds of this type it is essential to define the relative contribution of each component to the overall pharmacological profile of the compound. Whilst in vitro profiling can provide some insights on this subject, it cannot be used as a definitive representation of a compound’s actions in vivo.

In this study, we have explored whether binding site occupancy measured ex vivo by radioligand binding will provide a viable technique for estimating a compound’s likely in vivo efficacy across a range of its potential molecular targets. For this evaluation, we have profiled the relative occupancy of the noradrenaline, dopamine and 5-HT reuptake transporters by various reference reuptake inhibitors, ie atomoxetine, duloxetine and nomifensine. The pharmacological profile of these drugs has been compared with their relative potencies as inhibitors of the synaptosomal uptake of the monoamines in vitro.

The monoamine reuptake inhibitors were chosen as the class of drugs to validate the ex vivo occupancy model because of the renewed interest in them as therapeutic agents as evidenced by the recent regulatory approval of atomoxetine for the treatment of ADHD and duloxetine for depression.

**METHODS**

**MONOAmine UPTAKE STUDIES**

Inhibition of \[^3^H\]NA (10nM), \[^3^H\]5-HT (2nM) and \[^3^H\]DA (2.5nM) uptake into cortical noradrenaline (NA) and 5-HT and striatal dopamine (DA) synaptosomes was determined. Frontal cortex (for 5-HT and NA assays) and striata (for DA assays) were dissected from male Sprague-Dawley rats (250-300g). Synaptosomes were prepared by homogenisation and centrifugation. Non-specific uptake was defined by zimeldine (10µM), desipramine (10µM) and GBR 12909 (10µM). Uptake was terminated by filtration under vacuum using a Skatron cell harvester, through Skatron 11734 filters and radioactivity determined by liquid scintillation counting (1 ml Packard MV Gold Scintillator).

**EX VIVO RADIOLIGAND BINDING STUDIES**

Male Sprague-Dawley rats (200-250g) were given vehicle or drug (10mg/kg po). One hour later whole brains were removed, frontal cortex and striata dissected and frozen at -20°C until required. On the day of assay membranes were prepared and binding to frontal cortical noradrenaline (NA) and 5-HT and striatal dopamine (DA) uptake sites were determined using \[^3^H\] nisoxetine (0.6nM), \[^3^H\] citalopram (1.3nM) and \[^3^H\] WIN 35428 (5nM). Non-specific binding was defined by paroxetine (0.5µM), mazindol (1µM) and GBR 12936 (1µM). Binding was terminated by filtration under vacuum using a Skatron cell harvester, through Skatron 11734 filters and radioactivity determined by liquid scintillation counting (1 ml Packard MV Gold Scintillator).

**RESULTS**

Table 1. Pharmacological profiles of the reference monoamine reuptake inhibitors in vitro

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Ki value (nM)</th>
</tr>
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<tbody>
<tr>
<td>NA</td>
<td>5-HT</td>
</tr>
<tr>
<td>Atomoxetine</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Duloxetine</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>Nomifensine</td>
<td>12.9 ± 2.9</td>
</tr>
</tbody>
</table>

Ki values for the inhibition of tritiated NA, DA or 5-HT uptake into rat brain synaptosomes. n=3-4.

**SUMMARY AND CONCLUSIONS**

Ex vivo binding studies to determine the occupancy of the monoamine reuptake transporters by these reference drugs yielded the following results:

- Atomoxetine, duloxetine and nomifensine each achieved approximately equal levels of occupancy of NA reuptake sites at 10 mg/kg po, ie 85%, 76% and 90% respectively.
- Atomoxetine (10 mg/kg po) occupied 85% of NA reuptake sites with no significant occupancy for those of DA or 5-HT.
- Duloxetine (10 mg/kg po) showed comparable potencies to occupy NA and 5-HT reuptake sites, ie 85% and 70%, respectively, but did not bind to the dopamine reuptake transporter.
- In spite of being the drug with the lowest potency in vitro, nomifensine (10 mg/kg po) nevertheless occupied almost all (90%) of the NA reuptake sites in vitro. It also occupied 35% of the DA uptake sites, but did not bind to the 5-HT transporter.
- These findings are generally consistent with in vitro reuptake inhibition data, but some interesting subtle differences were observed.
- Close congruence between the in vitro and ex vivo data in this study was anticipated because both sets of experiments were performed using native transporters in physiologically relevant environments. However, where drug profiling in vitro has been performed using cloned/transfected targets in cell lines, a much wider divergence between the in vitro and ex vivo data is likely.

**Ex vivo radioligand binding is, therefore, a valuable technique for defining the pharmacological actions of a drug on multiple molecular targets in vivo.** This method can be used either to profile therapeutic actions across a range of receptors/binding sites, or alternatively, to define the therapeutic window for compounds which also have affinities for receptors or binding sites linked to clinical side-effects or adverse events.