Evaluation of the Monoamine Transporter Activities of the New Psychoactive Substances (NPS) 4,4'-dimethylaminorex (4,4'-DMAR) and 3',4'-methylenedioxy-4-methylaminorex (MDMAR)

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Introduction

In recent times, increasing political, media, and public attention have been focused on the emergence of new psychoactive substances (NPS). These substances are being abused recreationally and have been growing in popularity at an unprecedented rate over the last number of years. Currently, the number of NPS (348) exceeds the total number of substances listed under international control (234). The majority of these drugs are released onto the market with no chemical or pharmaceutical data available. Thus, their unknown pharmacological effects, routes of administration, and potential potency, can pose serious risks to users.

Between the second half of 2013 and early 2014, a total of 31 deaths involving the new psychostimulant cis-4,4'-dimethoxyaminoamphetamine (4,4'-DMAR) were reported to the European Monitoring Centre for Drugs And Drug Addiction (EMCDDA). Subsequently, the existence of 3',4'-methylenedioxy-4-methylaminorex (MDMAR) has come to the authors' attention. The parent compound aminorex and its analogue 4-methoxyamphetamine (MDAR) are known psychostimulants, both first synthesized in the 1960s and evaluated as potential appetite suppressants.

This study describes the synthesis of cis- and trans-MDMAR followed by extensive analytical characterisation by chromatographic, spectroscopic, mass spectrometric platforms and crystal structure analysis. Monoamine release activities of both MDMAR isomers and DMAR isomers were compared with the non-selective monoamine releasing agent (±)-3,4-methylenedioxymethylamphetamine (MDMA) as a standard reference compound.

Synthesis and Characterisation

The synthesis procedure (Figure 2) for cis- and trans-MDMAR was essentially adapted from the method previously reported by Brandt et al. for the preparation of cis- and trans-4,4'-DMAR isomers. In this case, the starting material used was 3',4'-methylenedioxynorephedrine.

Monoamine Transporter Activity

At present, the biological mechanism of action of both 4,4'-DMAR and MDMAR isomers has not been fully elucidated. Drugs with similar amphetamine-like structures, such as aminorex, are known to act as substrates for monoamine transporter proteins, thereby leading to release of monoamine neurotransmitters – dopamine, serotonin and noradrenaline – in the central nervous system. In this study, the monoamine release activities of both MDMAR isomers were compared with that of both 4,4'-DMAR and the reference compound compound.

Release Assay Method

Male Sprague-Dawley rats (250-300g, Charles Laboratories, Wilmington, MA, USA) were euthanized by CO2 narcosis and brains were processed to yield nerve terminals from the rat brain.

For the release assay, 9mM of [5H]-L-methylphenylephrine ([5H]MP) was used as the radiolabelled substrate for dopamine transporters (DAT) and noradrenaline transporters (NET), whereas 50µM of [3H]-norephedrine ([3H]NE) was used as the radiolabelled substrate for 5-HT transporters (SERT). All buffers contained 1µM reserpine to block vesicular uptake of substrates. The selectivity of release assays was optimised for a single transporter by including unlabelled blockers to prevent the uptake of [5H]MP or [3H]NE by competing transporters. Sypmptomes were preloaded with radiolabelled substrate in Krebs-phosphate buffer for 1 hour. Release assays were initiated by adding 850 µL of preloaded symptomes to 150 µL of test drug. Release was terminated by vacuum filtration and retained radioactivity was quantified by scintillation counting.

Results

Figure 4 shows the dose response effects of cis-DMAR, trans-DMAR, cis-MDMAR and trans-MDMAR on transmitter release at DAT, NET and SERT.

Both isomers of MDMAR and a vendor sample were subjected to extensive analytical characterisation by chromatographic, spectroscopic, mass spectrometric platforms and crystal structure analysis.

monoamine transport receptor activity

The high potency of the ring substituted methylaminorex analogues and their ability to be fully efficacious substrate-type releasers might contribute to the possibility of a range of serious side effects after high dose exposure and/or when combined with other substances that act on similar targets. Psychotic symptoms, agitation and paranoia is a result from overstimulation of central dopamine and 5-HT systems, whereas dangerous cardiovascular effects could be produced by excessive noradrenaline release in the periphery. This was the first report on the characterisation of MDMAR, which demonstrates the continuous need to remain vigilent on the availability of newly emerging psychoactive substances.

Table 1 summarises potency values at concentrations producing 50% of maximal release (EC50) for the test drugs based on data depicted in Figure 4.

Table 1. Summary of potency values (EC50) for the test drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Release at DAT EC50 (nM)</th>
<th>Release at NET EC50 (nM)</th>
<th>Release at SERT EC50 (nM)</th>
<th>DAT/SERT Ratio</th>
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| (+)-DMAR | 143.6 ± 16.0             | 98.3 ± 15.0              | 85.0 ± 13.3               | 250 ± 61,1 |-DAT/SERT ratio calculated by ([EC50 at DAT]/[EC50 at SERT])
| cis-DMAR | 10.9 ± 0.7               | 11.8 ± 2.0               | 17.7 ± 2.3                | 1.6            |
| trans-DMAR | 24.4 ± 2.7             | 31.6 ± 4.6               | 59.9 ± 17.2               | 2.5            |
| cis-MDMAR | 10.2 ± 1.2             | 14.8 ± 2.7               | 43.9 ± 6.7                | 4.3            |
| trans-MDMAR | 36.2 ± 3.6             | 38.9 ± 4.7               | 73.4 ± 12.0               | 2.0            |

* Data are expressed as mean ± SD for N=3-4 experiments performed in triplicate.

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References


