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)

G McLaughlin[1,2], N Morris[1], PV Kavanagh[2], JD Power[2,3], B Twamley[4], J O'Brien[5], B Talbot[6], G Dowling[7], O Mahony[8], SD Brandt[9], J Patrick[10], RA Archer[11], JS Partilla[12] and MH Baumann[12]

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 detected (348) exceeds the total number of substances under international control (234). The majority of these drugs are released onto the market with no chemical or pharmacological data available. Thus, their unknown pharmacological effects, routes of administration, and potential potency, can pose serious risks to users.

4,4'-DMAR and MDMAR

Between the second half of 2013 and early 2014, a total of 31 deaths involving the new psychostimulant *cis*-4,4'-dimethylaminorex (4,4'-DMAR) were reported to the European Monitoring Centre for Drugs And Drug Addtion (EMCDDA).[1] Subsequently, the existence of 3',4'-methylenedioxy-4-methylaminorex (MDMAR) has come to the authors' attention. The parent compound aminorex and its analogue 4-methylaminorex (4-MAR) are known psychostimulants, both first synthesized in the 1960s and evaluated as potential appetite suppressants.

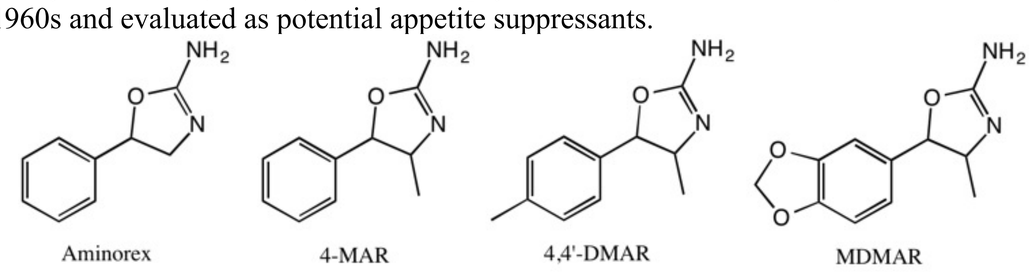

Figure 1. Chemical structures of aminorex and its analogues

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-methylenedioxymethamphetamine (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.[2] In this case, the starting material used was 3',4'-methylenedioxynorephedrine.

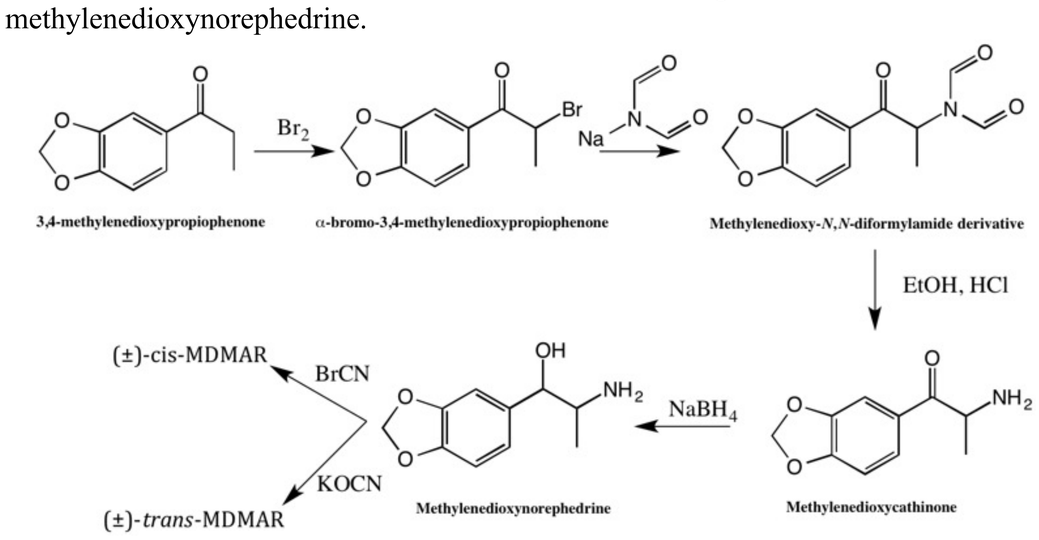

Figure 2. Synthesis of *cis*- and *trans*-MDMAR

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 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 norepinephrine – in the central nervous system.[3,4] In this study, the monoamine release activities of both MDMAR isomers were compared with that of both 4,4'-DMAR isomers and the reference compound MDMA.

Release Assay Method

Male Sprague-Dawley rats (250-300g, Charles Laboratories, Wilmington, MA, USA) were euthanised by CO_2 narcosis and brains were processed to yield synaptosomes.[5,6] For the release assay, 9nM [^{3}H]-1-methyl-4-phenylpyridinium ([^{3}H]MPP$^+$) was used as the radiolabelled substrate for dopamine transporters (DAT) and norepinephrine transporters (NET), while 5 nM [^{3}H]5-HT was used as the radiolabelled substrate for 5-HT transporters (SERT).

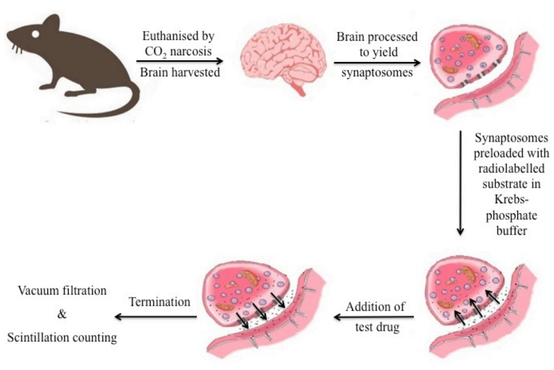

Figure 3. Schematic representation of the Release Assay Method

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 [^{3}H]MPP$^+$ or [^{3}H]5-HT by competing transporters. Synaptosomes were preloaded with radiolabelled substrate in Krebs-phosphate buffer for 1 hour. Release assays were initiated by adding 850 μL of preloaded synaptosomes 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.

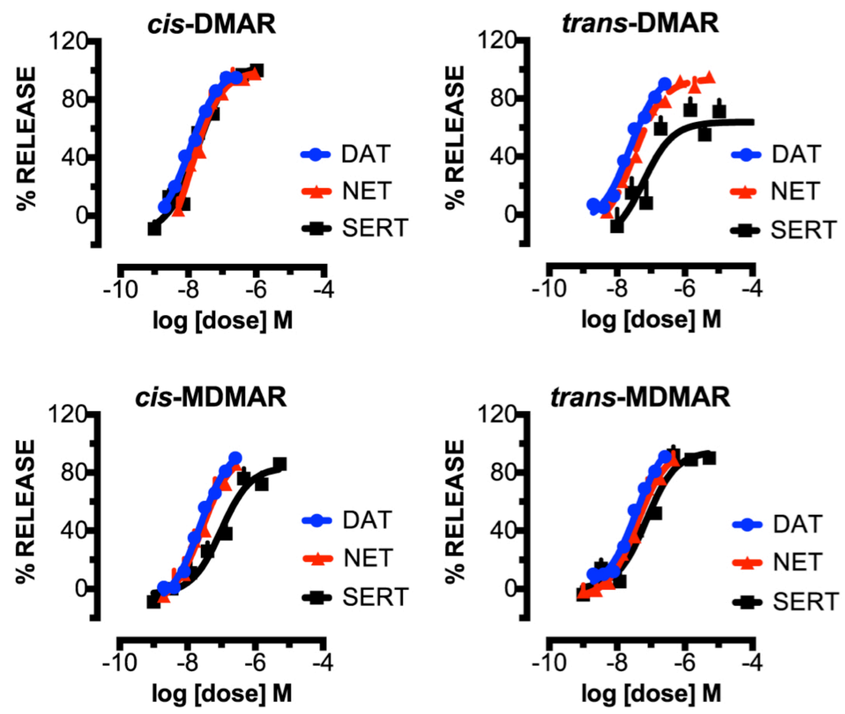

Figure 4. Dose response effects of *cis*-DMAR, *trans*-DMAR, *cis*-MDMAR and *trans*-MDMAR on transmitter release at DAT, NET and SERT.

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

Table 1. Summary of potency values (EC_{50}) for the test drugs

Drug	[a] Release at DAT EC_{50} (nM)	[a] Release at NET EC_{50} (nM)	[a] Release at SERT EC_{50} (nM)	[b] DAT/SERT Ratio
(+)-MDMA	143.0 ± 16.0	98.3 ± 15.0	85.0 ± 13.3	0.6
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

[a] Data are expressed as mean ± SD for N=3-4 experiments performed in triplicate
[b] DAT/SERT ratio calculated by $(EC_{50}$ at DAT$)^{-1}/(EC_{50}$ at SERT$)^{-1}$; higher value indicates greater DAT selectivity

cis-DMAR, *trans*-DMAR, *cis*-MDMAR and *trans*-MDMAR were potent and efficacious substrate-type releasers at DAT and NET in rat brain tissue. *cis*-DMAR, *cis*-MDMAR and *trans*-MDMAR were fully efficacious releasing agents at SERT as well, while *trans*-DMAR displayed partial releasing activity at this transporter. It is hypothesised that *trans*-DMAR displays the unusual profile of a catecholamine releaser with 5-HT uptake blocking properties. The potencies for *cis* and *trans* racemates of DMAR and MDMAR were generally similar at each transporter, indicating a minimal influence of stereoselectivity in determining drug transporter interactions across a group of structures. All of the 4-methylaminorex analogues tested here had a profile of transporter releasing activity that mimics the effects of MDMA, which is a non-selective transporter releaser, but the 4-methylaminorex analogues were more potent.

Conclusion

The high potency of the ring subsituted 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 hyperthermia could result from overstimulation of central dopamine and 5-HT systems, whereas dangerous cardiovascular effects could be produced by excessive norepinephrine release in the periphery. This was the first report on the characterisation of MDMAR, which demonstrates the continuous need to remain vigilant on the availability of newly emerging psychoactive substances.

References

1. Risk assessment report of a new psychoactive substance:4-methyl-5-(4-methylphenyl)-4,5-dihydrooxazol-2-amine (4,4′-dimethylaminorex, 4,4′-DMAR). In accordance with Article 6 of Council Decision 2005/387/JHA on information exchange, risk assessment and control of new psychoactive substances. EMCDDA, Lisbon, 2014.
2. S.D. Brandt, M.H. Baumann, J.S. Partilla, P.V. Kavanagh, J.D. Power, B. Talbot, B. Twamley, J. O'Brien, O. Mahony, S.P. Elliott, R.P. Archer, J. Patrick, K. Singh, N.M. Dempster, S.H. Cosbey. Characterization of a novel and potentially lethal designer drug, (±)-cis-para-methyl-4-methylaminorex (4,4′-DMAR, or 'Serotoni'). Drug Test. Anal. 2014, 6, 684.
3. R.B. Rothman, M.H. Baumann. Monoamine transporters and psychostimulant drugs. Eur. J. Pharmacol. 2003, 479, 23.
4. T. Hofmaier, A. Luf, A. Seddik, T. Stockner, M. Holy, M. Freissmuth, G.F. Ecker, R. Schmid, H.H. Sitte, O. Kudlacek. Aminorex, a metabolite of the cocaine adulterant levamisole, exerts amphetamine like actions at monoamine transporters. Neurochem. Int. 2014, 73, 32.
5. R.B. Rothman, N. Vu, J.S. Partilla, B.L. Roth, S.J. Hufeisen, B.A. Compton-Toth, J. Birkes, R. Young, R.A. Glennon. In vitro characterization of ephedrine-related stereoisomers at biogenic amine transporters and the receptorome reveals selective actions as norepinephrine transporter substrates. J. Pharmacol. Exp. Ther. 2003, 307, 138.
6. M.H. Baumann, M.A. Ayestas Jr, J.S. Partilla, J.R. Sink, A.T. Shulgin, P.F. Daley, S.D. Brandt, R.B. Rothman, A.E. Ruoho, N.V. Cozzi. The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. Neuropsychopharmacology 2012, 37, 1192.

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Affililations : **1.** Department of Life and Physical Sciences, School of Science, Athlone Institute of Technology, Co. Westmeath, Ireland. **2.** Department of Pharmacology & Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland. **3.** Forensic Science Laboratory, Garda Headquarters, Dublin 8, Ireland. **4.** TCD Small Molecule X-Ray Facility, Trinity College Dublin, Dublin 2, Ireland. **5.** School of Chemistry, Trinity College Dublin, Dublin 2, Ireland. **6.** School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland. **7.** The State Laboratory, Backweston Laboratory Complex, Young's Cross, Celbridge, Co. Kildare, Ireland. **8.** School of Chemical and Pharmaceutical Sciences, Dublin Institute of Technology, Dublin 2, Ireland. **9.** School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L33, UK. **10.** Scientific Supplies Ltd, Hanover Street, London W1S 1YH, UK. **11.** States Analyst's Laboratory, Longue Rue, St. Martin's, GY4 6LD, Guernsey. **12.** Designer Drug Research Unit of the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, USA