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MEETING ABSTRACT Open Access

4-Methylthioamphetamine (4-MTA) induces mitochondrial-dependent apoptosis in SH-SY5Y cells independently of dopamine and noradrenaline transporters

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Background

3,4-Methylenedioxymethamphetamine (MDMA or 'ecstasy') tablets are frequently contaminated by 4-MTA ('flatliner'), an amphetamine derivative which is known to induce severe human intoxication and even death. Although an equipotent inducer of SERT-dependent 5-HT release *in vivo*, 4-MTA does not induce MDMA-like serotoninergic neurotoxicity in rats. Instead, 4-MTA users typically report unpleasant sympathomimetic effects such as tachycardia, tremors, stomach cramps, headache and sweating following ingestion. Here, for the first time we investigate the cytotoxic potency of 4-MTA in a catecholaminergic system.

Methods

SH-SY5Y cells express both dopamine and noradrenaline transporters (DAT, NET) in the presence of vesicular monoamine transporter 2 (VMAT2) and were therefore chosen as the ideal catecholaminergic model in which to examine the molecular mechanisms of 4-MTA and MDMA-induced cytotoxicity *in vitro*. Cell viability was determined using the MTT assay and validated using flow cytometry via PI exclusion. ROS production, mitochondrial membrane potential (MMP), apoptosis and the cell cycle were examined via flow cytometry using $DCFH_2DA$, JC-1, annexin V/PI and PI respectively. The level of intracellular calcium was determined ratiometrically by confocal microscopy using two visible wavelength Ca^{2+}-sensitive dyes, Fluo-3 and Fura Red.

Results

4-MTA was significantly more cytotoxic than MDMA at 24 h, demonstrating an EC_{50} of 0.60 mM in contrast to 2.01 mM for MDMA. In addition, the combination of MDMA and 4-MTA at low concentrations significantly increased cytotoxicity compared to that of each drug alone. 4-MTA-induced cell death was reduced by the anti-oxidant *N*-acetyl-L-cysteine (NAC) but not by the non-selective monoamine transport inhibitor indatraline, indicating that monoamine transport is not a requirement of 4-MTA-induced cytotoxicity. Drug-induced cell death was pre-empted by rapid intracellular Ca^{2+} influx, mitochondrial membrane depolarization (MMD), ROS production and caspase 9 activation. MDMA and 4-MTA also induced phosphatidlyserine exposure and caspase-dependent DNA fragmentation at 24 h indicative of cell death via apoptosis.

Conclusions

Although both MDMA and 4-MTA induced apoptosis via the mitochondrial death pathway, 4-MTA does so at more physiologically relevant concentrations and may therefore be a potent synergistic adjunct when mixed with MDMA.

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