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Actions of amphetamine derivatives and cathinone at the noradrenaline transporter

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Abstract

We have recently shown that methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), cathinone and methylenedioxyethylamphetamine (MDEA) have a cocaine-like action to potentiate the contractile actions of noradrenaline but not isoprenaline in the 1-Hz paced rat right ventricle. The purpose of this study was to directly test the actions of these compounds at the noradrenaline transporter. In rat left ventricular slices, potency ($-\log IC_{50}$) values at inhibiting uptake of [^{3}H]noradrenaline were: cocaine 6.16 ± 0.15, cathinone 6.03 ± 0.16, MDMA 6.05 ± 0.07, MDA 5.68 ± 0.06 and MDEA 5.56 ± 0.08. MDEA and MDA were significantly less potent. In rat cerebral cortex membranes, MDMA was significantly less potent at displacing [^{3}H]nisoxetine binding; $-\log EC_{50}$ values: cocaine 5.04 ± 0.08, cathinone 5.40 ± 0.14, MDA 4.66 ± 0.11, MDEA 4.99 ± 0.15, MDMA 4.22 ± 0.07. The noradrenaline uptake studies showed that MDEA was least potent: MDEA was also least potent functionally in the paced rat right ventricle. The [^{3}H]nisoxetine displacement studies did not compare with the functional studies.

Keywords: MDMA (methylenedioxymethamphetamine); MDA (methylenedioxyamphetamine); MDEA (methylenedioxyethylamphetamine); Cathinone; Cocaine; Nisoxetine

1. Introduction

Amphetamine derivatives such as MDMA (3,4-methylenedioxymethamphetamine), MDEA (3,4-methylenedioxyethylamphetamine) and MDA (3,4-methylenedioxyamphetamine) are now widely abused as recreational drugs resulting in fatalities, but their toxicity has been much less studied than that of cocaine and classical amphetamines. MDMA is reported to have cardiac stimulant actions in rats resulting in tachycardia and arrhythmias (Gordon et al., 1991) and is also reported to facilitate vasoconstriction in the rat (FitzGerald and Reid, 1994). Tachycardia and hypertension (Hayner and McKinney, 1986), and cardiovascular mortality (Dowling et al., 1987) have been reported in man. In addition, MDMA has been linked to intracerebral haemorrhage (Harries and De Silva, 1992), and cerebral hyperperfusion to MDMA can be demonstrated in rats (Kelly et al., 1994). Chronic use of another amphetamine derivative, methamphetamine, has been shown to result in serious cardiovascular changes including tachycardia and palpitations (Chan et al., 1994). Fenfluramine, also an amphetamine derivative, has been linked to valvular heart disease and this may involve the serotonin transporter in the heart (Connolly et al., 1997; Brouri et al., 2002). Cathinone, the active constituent of widely used *Khat* has amphetamine-like actions (Kalix and Glennon, 1986) and has recently been shown to be a risk factor for myocardial infarction (Al-Motarreb et al., 2002).

We have recently shown that MDMA, MDA, cathinone and to a lesser extent MDEA share with cocaine an ability to potentiate the contractile actions of noradrenaline but not isoprenaline in the 1-Hz paced rat right ventricle (Cleary et al., 2002). It was concluded that these drugs, like cocaine, prevented the re-uptake of noradrenaline into nerve terminals, an action which could contribute to the cardiovascular complications associated with MDMA abuse. The purpose of this study was to verify the above hypothesis by directly testing the actions of these compounds at the noradrenaline transporter.

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2. Methods

Male Wistar rats (250–350 g) were obtained from Trinity College Dublin. The studies conform to the Declaration of Helsinki and have been approved by the Department of Health and by the RCSI Research Ethics Committee.

2.1. Inhibition of [^{3}H]noradrenaline accumulation

Inhibition of noradrenaline accumulation was determined according to the methods described by Leineweber et al. (2000). Rats were killed by CO_2 overdose and their hearts were quickly removed. Inhibition of [^{3}H]noradrenaline (49.7 Ci/mmol, NEN) uptake was measured as follows. Left ventricular slices of approximately 10 mg were incubated for 15 min at 37 °C in oxygenated, modified Krebs-Henseleit solution (composition, mM: NaCl, 118; $NaHCO_3$, 25; D-glucose, 11; KCl, 4.7; $CaCl_2$, 2.5; ascorbic acid, 1.14; $NaHPO_4$, 1; $MgCl_2$, 0.54; Pargyline, 0.120; EDTA, 0.094) with [^{3}H]noradrenaline (25 nM) and increasing concentrations of amphetamine derivative. Nonspecific uptake was determined by parallel incubation at 4 °C. The assays were terminated by vacuum filtration through Whatman GF/C glass fibre filters presoaked in 0.05% ice-cold polyethyleneimine solution. Filters were washed three times with 2 ml of ice-cold modified Krebs-Henseleit solution. Tissue slices and filters were transferred to scintillation vials containing 2 ml ice-cold 3% trichloroacetic acid solution. Following overnight incubation at 4 °C, scintillation fluid was added to the vials before counting. Total tritium was taken as a measure of [^{3}H]noradrenaline accumulation.

2.2. Displacement of [^{3}H]nisoxetine binding

Displacement of [^{3}H]nisoxetine (81.8 Ci/mmol, NEN) binding to the Noradrenaline transporter was measured using a method adapted from that described by Tejani-Butt et al. (1991). Rats were killed by CO_2 overdose. Brains were quickly removed and placed on ice. Cerebral cortices were carefully removed and homogenised in 30 volumes of ice-cold wash buffer (Composition: NaCl, 120 mM; Tris–HCl, 50 mM; KCl, 5 mM: pH 7.4 at 4 °C). The homogenate was centrifuged at 14,000 rpm for 15 min at 4 °C. The supernatent was discarded, the pellet resuspended and homogenization and centrifugation steps were repeated twice. The pellet was then suspended in 30 volumes of incubation buffer (NaCl, 300 mM; Tris–HCl, 50 mM; KCl, 5 mM: pH 7.4 at 4 °C) homogenized. Aliquots of rat cerebral cortex membranes were incubated for 4 h at 4 °C with 2 nM [^{3}H]nisoxetine and increasing concentrations of amphetamine derivative. Nonspecific binding was determined in the presence of desipramine (0.3 μM). The assay was terminated by addition of 4 ml ice-cold incubation buffer. This was followed by rapid filtration through Whatman GF/B filters, which were presoaked in 0.05% polyethyleneimine solution. Filters were washed three times with 5 ml ice-cold incubation buffer and then transferred to a scintillation vial. Scintillation fluid was added and vials were left overnight before counting.

2.3. Statistics

Values are expressed as mean and standard error of the mean (S.E.M.). pD_2 ($-\log EC_{50}$) values were calculated using the GraphPad Prism programme for PC. Differences between groups and vehicle were compared, using the Instat programme for Macintosh, by Student's *t*-test for paired or unpaired data, where appropriate, and by Analysis of Variance with Dunnett's test for comparison of effects of vehicle with test drug, or Tukey's test for comparison of all groups. Means were considered significantly different when P values were <0.05.

2.4. Drugs

Cathinone (Sigma); (−)-isoprenaline hydrochloride (Sigma); methylenedioxyamphetamine (MDA: Sigma); methylenedioxyethylamphetamine (MDEA: Sigma); methylenedioxymethamphetamine (MDMA: Research Biochemicals); (−)-noradrenaline hydrochloride (Sigma); pargyline (Sigma).

All drugs were dissolved in distilled water.

3. Results

3.1. Inhibition of [^{3}H]noradrenaline accumulation

Total specific [^{3}H]noradrenaline uptake (measured as total tritium) was 43.46 ± 2.65 fmol/mg/15 min ($n=24$). Cocaine, MDMA, cathinone, MDA and MDEA all inhibited

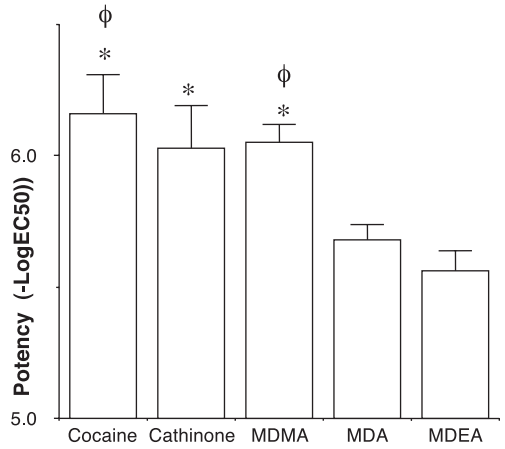

Fig. 1. Effects of cocaine MDMA, cathinone, MDEA and MDA on accumulation of [^{3}H]noradrenaline in segments of rat left ventricle, expressed as a pD2 (− log IC50 for inhibition of noradrenaline accumulation). Vertical bars represent S.E.M. from five experiments. *Denotes significantly more potent than MDEA. $^{\phi}$Denotes significantly more potent than MDA ($^{\phi}$,*$P<0.05$; Analysis of Variance and Tukey's test).

uptake of [^{3}H]noradrenaline into rat left ventricular slices. Potency ($-\log EC_{50}$) values were: cocaine 6.16 ± 0.15, cathinone 6.03 ± 0.16, MDMA 6.05 ± 0.07, MDA 5.68 ± 0.06 and MDEA 5.56 ± 0.08 (Fig. 1). Cocaine, cathinone and MDMA were significantly more potent than MDEA. MDA was significantly less potent than MDMA and cocaine.

3.2. Displacement of [^{3}H]nisoxetine binding

Cocaine, MDMA, Cathinone, MDA and MDEA all displaced [^{3}H]nisoxetine binding at the rat cerebral cortex noradrenaline transporter. Potency ($-\log EC_{50}$) values were: Cocaine 5.04 ± 0.08, Cathinone 5.40 ± 0.14, MDA 4.66 ± 0.11, MDEA 4.99 ± 0.15, MDMA 4.22 ± 0.07 (Fig. 2). MDA was significantly less potent than cocaine and cathinone. MDMA was significantly less potent at displacing [^{3}H]nisoxetine binding than cocaine, cathinone, MDEA and MDA.

3.3. Comparison with functional data obtained in 1-Hz paced rat right ventricle

In rat right ventricular strips paced at 1 Hz, we have previously shown that cocaine, MDMA, MDA, MDEA and cathinone significantly increase the potency of noradrenaline (Cleary et al., 2002). The rank order of potency in paced right ventricle, in which MDMA was most potent and MDEA least potent, fits better with the inhibition of [^{3}H]noradrenaline accumulation than with inhibition of [^{3}H]nisoxetine binding. MDEA was significantly less potent than the other agents in paced right ventricle (see Cleary et al., 2002), which is in agreement with the results of the [^{3}H]noradrenaline accumulation studies but not with the [^{3}H]nisoxetine binding studies.

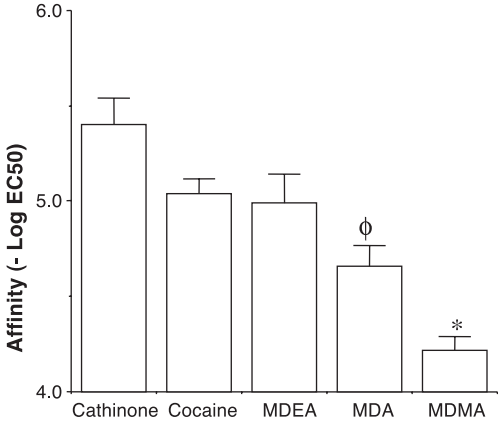

Fig. 2. Displacement of [^{3}H]nisoxetine binding in rat cortical membranes by cocaine MDMA, cathinone, MDEA and MDA, expressed as a pD2 ($-\log IC50$ for displacement of nisoxetine binding). Vertical bars represent S.E.M. from five experiments. *Denotes significantly less potent than every other test drug. $^{\phi}$Denotes significantly less potent than cathinone and cocaine ($^{\phi}$,*$P < 0.05$; Analysis of Variance and Tukey's test).

4. Discussion

In this study, we have investigated the effects of the amphetamine-like agents MDMA, MDA (a metabolite of MDMA), MDEA ('Eve') and cathinone (from khat) in comparison to cocaine at the noradrenaline transporter and compared the results to their effects on contractile responses induced by noradrenaline in paced rat right ventricle.

The functional studies of Cleary et al. (2002) in paced rat right ventricle were verified by the results of the noradrenaline uptake studies which showed that MDMA, cathinone, MDA and MDEA all inhibit uptake of noradrenaline in the rat left ventricle. MDEA was the least potent and this reflects its lesser ability to potentiate noradrenaline in the paced rat right ventricle.

Results of the [^{3}H]nisoxetine displacement studies confirmed that all of the drugs tested bind to the noradrenaline transporter. However, here MDMA was significantly less potent than the other drugs and this does not compare with the functional studies. We can only assume that the discrepancies are due to differential functioning of the noradrenaline transporter with regards to binding and uptake of noradrenaline. Eshleman et al. (1999) also reported a poor correlation between inhibition of binding and inhibition of uptake for compounds at the noradrenaline transporter. Studies involving mutant and chimera transporters have shown that different domains of the noradrenaline transporter are involved in binding and translocation (Giros et al., 1994, Roubert et al., 2001); therefore, it is no surprise that discrepancies exist between binding affinities and uptake inhibition potencies of these compounds.

These results imply that the actions of these compounds are more cocaine-like than previously thought. It is possible that these compounds are not substrates but inhibitors of the noradrenaline transporter, which also have the ability to passively enter the nerve terminal and cause carrier-mediated release of noradrenaline. Both Schmidt et al. (1987) and Wang et al. (1987) found little evidence for carrier-mediated uptake of [^{3}H]MDMA into synaptosomes, suggesting that MDMA probably enters the neuron by passive mechanisms to cause the carrier-mediated release/displacement of neurotransmitter.

In our studies, test agents have pharmacological actions at concentrations of approximately 1 μM. Typical human illegal and/or pharmacokinetic doses of MDMA are 1–2 mg/kg or 4–8 μmol/kg (O'Loinsigh and O'Boyle, 1998; Mas et al., 1999; De La Torre et al., 2000). Peak plasma levels of MDMA following 125 mg were 1.07 μM (Mas et al., 1999), and of cathinone following khat 0.8 mg/kg were 0.7 μM (Widler et al., 1994). Hence, the effects seen in our studies occur in the range of concentrations expected in man. Since cocaine is known to increase acutely the risk of myocardial infarction (Mittleman et al., 1999), and at a dose of 1 mg/kg (2.9 μmol/kg) causes cerebrovascular abnormalities (Johnson et al., 1997), it might be expected that MDMA, MDA, cathinone and to a lesser extent MDEA,

have similar actions both in terms of ventricular and vasoconstrictor actions. Cocaine, by blocking the noradrenaline transporter, and MDMA, MDA, MDEA and cathinone by blocking the transporter and by displacing noradrenaline were able to produce similar potentiations of the contractile actions of noradrenaline in rat ventricle. These findings do not mean that MDEA is in anyway a safer drug than the other amphetamine derivatives.

In conclusion, cocaine, MDMA, MDA, cathinone and to a lesser extent, MDEA inhibit the uptake of noradrenaline by an action which involves competitive blockade of the noradrenaline transporter. This action may contribute to cardiac morbidity as previously shown for cocaine.

Acknowledgements

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