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In vitro neuronal and vascular responses to 5-hydroxytryptamine: modulation by 4-methylthioamphetamine, 4-methylthiomethamphetamine and 3,4-methylenedioxymethamphetamine

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Abstract

4-Methylthioamphetamine and 4-methylthiomethamphetamine are thioarylethylamines structurally related to 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'). This study compared effects of these agents and MDMA on 5-hydroxytryptamine (5-HT) signalling systems in the brain and vasculature in vitro. Both 4-methylthioamphetamine and 4-methylthiomethamphetamine (100 μM) reduced the rate of specific high affinity [^{3}H]5-HT reuptake in isolated rat brain synaptosomes to 14% and 10% of control, respectively. The concentration required for half-maximal inhibition (IC_{50}) of [^{3}H]5-HT reuptake by 4-methylthioamphetamine (0.27 μM) was significantly lower ($P<0.005$) than that for inhibition by MDMA (1.28 μM) and that for inhibition by 4-methylthiomethamphetamine (0.89 μM). Both 4-MTA and 4-MTMA caused a significant release of preloaded [^{3}H]5-HT from synaptosomes, but were significantly less effective than MDMA at the concentrations tested (1–100 μM). In isolated rat aorta, a 15-min preincubation with 4-methylthioamphetamine or 4-methylthiomethamphetamine significantly reduced the maximal contraction (E_{max}) induced by 5-HT to 71% or 91% of control ($P<0.05$ in each case), respectively. In addition, 4-methylthiomethamphetamine (100 μM), but not 4-methylthioamphetamine, significantly increased the concentration of 5-HT required for half-maximal contraction (EC_{50}) from 4.13 to 20.08 μM ($P<0.0001$). In contrast, MDMA did not significantly alter the E_{max} or the EC_{50} of 5-HT-induced aortic contraction. It is concluded that both 4-methylthioamphetamine and 4-methylthiomethamphetamine are potent inhibitors of [^{3}H]5-HT reuptake in the brain. Furthermore, unlike MDMA, they both directly inhibit 5-HT-mediated vascular contraction. These results suggest that these compounds may be potentially more harmful than MDMA in the context of human misuse.

Keywords: 4-MTA (4-methylthioamphetamine); 4-MTMA (4-methylthiomethamphetamine); MDMA (3,4-methylenedioxymethamphetamine); 5-HT (5-hydroxyhyptamine, serotonin); Reuptake; Synaptosome; Aortic contraction

1. Introduction

4-Methylthiomethamphetamine (4-MTA) has recently emerged as a 3,4-methylenedioxymethamphetamine (MDMA)-like drug of abuse, and was classified as a Schedule 1 controlled substance in 1999 (Groombridge, 1998; Poortman and Lock, 1999). Five deaths involving 4-MTA were recorded in the European Union in that year, although in all but one case, other agents (MDMA, amphetamines, alcohol and methadone) were detected in 4-MTA-containing samples (EMCDDA, 1999; Elliott, 2000). 4-MTA has also been identified as a constituent of a so-called herbal stimulant (De Boer et al., 1999).

The major acute neurochemical effects of 4-MTA in the rat are an increase in 5-hydroxytryptamine (5-HT) release and an inhibition of 5-HT reuptake, as well as inhibition of monoamine oxidase A (Scorza et al., 1999; Huang et al., 1992), which are all well-documented effects of MDMA (see review by Green et al., 1995). Unlike MDMA, however, 4-MTA reportedly lacks toxicity to serotonergic neurones, on the basis of a study showing that no long-term

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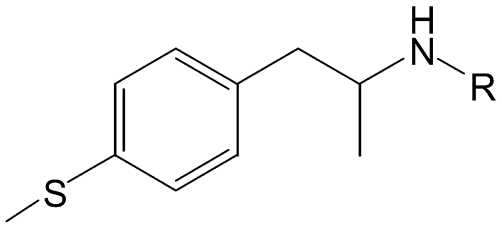

Fig. 1. The chemical structures of 4-methylthioamphetamine (R=H) and 4-methylthiomethamphetamine ($R=CH_3$).

depletion of 5-HT occurred following acute administration to rats (Huang et al., 1992). 4-Methylthiomethamphetamine (4-MTMA) has not been identified to date on the illicit market and there have been no reports of its effects on serotonergic neurones. However, due to its structural similarity to 4-MTA and because it is N-methylated, as is MDMA (Fig. 1), it is probable that 4-MTMA also targets 5-HT-releasing neurones.

The only reported cardiovascular effects of 4-MTA are a decrease in blood pressure and heart rate in rats treated with 5 mg/kg 4-MTA (Li et al., 1996). There are no reports to date of 4-MTMA actions in the cardiovascular system. MDMA, on the other hand, is known to induce cardiac arrhythmias (Dowling et al., 1987; Milroy et al., 1996), sudden cardiac death (Suarez and Riemersma, 1988), ventricular tachycardia, hypertension (Hayner and McKinney, 1986; Vollenweider et al., 1998; Mas et al., 1999) and impaired parasympathetic activity (Brody et al., 1998) in humans. In rats, MDMA has been reported to increase heart rate (Gordon et al., 1991). Interestingly, we have recently demonstrated impairment of a peripheral 5-HT-mediated response in rats chronically treated with MDMA (Cannon et al., 2001): twice daily administration of 20 mg/kg MDMA for up to 4 days resulted in reduced 5-HT-mediated contraction of aorta isolated 1 or 7 days later.

The aim of the present study was to increase knowledge of the structural specificity of the action of amphetamine-like compounds on 5-HT signalling systems in rat brain and vasculature. This was addressed by comparing the effects of 4-MTMA, 4-MTA and MDMA on the reuptake of 5-HT and release of preloaded [^{3}H]5-HT from rat brain synaptosomes, as well as on the responsiveness of isolated rat aorta to 5-HT.

2. Materials and methods

2.1. Synthesis of MDMA, 4-MTA and 4-MTMA

MDMA was synthesised by the method of Braun et al. (1980). In brief, isosafrole was oxidised to 3,4-methylenedioxyphenylacetone, which was then reacted with methylamine. The product of this reaction was treated with sodium cyanoborohydride to yield the corresponding MDMA derivative. 4-MTA was synthesised via $LiAlH_4$ reduction of the nitrostyrene derivative obtained from the nitroaldol/dehydration reaction of 4-methylthiobenzaldehyde and nitroethane (Butterick and Unrau, 1974). 4-MTMA was synthesised by a variation of the method of Butterick and Unrau (1974). MDMA, 4-MTA and 4-MTMA were prepared as their (±)-HCl salts. Their structures and purity (>99%) were confirmed by NMR, microanalysis and mass spectroscopy. All concentrations of MDMA, 4-MTA and 4-MTMA used refer to the (±)-HCl salt.

2.2. Synaptosomal preparation

Wistar rats (200–250 g) were killed by cervical dislocation and the brains quickly removed and placed in homogenisation medium at 4 °C containing 0.32 M sucrose, 1.0 mM EDTA and 0.25 mM dithiothreitol. Whole brain (minus the cerebellum) was homogenised and a synaptosomal fraction isolated by Percoll density gradient centrifugation, according to the method of Dunkley et al. (1988). The synaptosomal fraction was removed and washed twice in ice-cold Krebs' bicarbonate medium of the following composition (mM): NaCl 109.6, KCl 4.72, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, D-(+)-glucose 11 and $CaCl_2$ 2.5, pH 7.4, gassed with 95% O_2/5% CO_2, followed by centrifugation for 15 min at 16,000*g*. The final pellet, containing purified synaptosomes, was resuspended in Krebs' medium and maintained on ice until required.

2.3. Synaptosomal transport procedure

5-HT transport was initiated by the addition of 25 μl of the synaptosomal fraction to 975 μl of Krebs' bicarbonate medium, maintained at 37 °C, containing [^{3}H]5-HT ($4.0–6.0\times10^5$ Bq) diluted with unlabelled 5-HT to give a final concentration of 25 nM–1 μM (final specific activity 80–2400 Bq/pmol). Transport was arrested after 4 min by the addition of 200 μl of ice-cold 4 mM 5-HT and the tubes were immediately placed on ice prior to centrifugation for 10 min at 13,000*g* at 4 °C. The pellet was washed with homogenisation buffer and centrifuged as before. The final pellet was solubilised overnight in 2% sodium dodecyl sulphate and radiolabel incorporation was measured by liquid scintillation spectroscopy. Rates of transport are expressed as specific uptake of [^{3}H]5-HT (pmol/min/mg protein). This was determined by subtracting nonspecific transport (defined in the presence of 100 μM alaproclate) from total transport. 4-MTA, 4-MTMA or MDMA were added at time zero of the transport assay and measurements were performed on each synaptosomal fraction in triplicate. Protein was determined by the method of Markwell et al. (1978).

2.4. [^{3}H]5-HT release from synaptosomes

Release of preloaded [^{3}H]5-HT from crude synaptosomes was measured using the method of Rothman et al.

Table 1
Inhibition of [^{3}H]5-HT uptake by MDMA, 4-MTA and 4-MTMA

5-HT (nM)	Rate of transport (pmol/mg protein/min)		
	50	100	500
No drug	4.54±0.84	10.38±1.76	18.34±1.96
4-MTA (100 μM)	0.23±0.04*	0.55±0.19**	2.61±0.35***
4-MTMA (100 μM)	0.23±0.14*	1.02±0.26**	1.99±1.36***
MDMA (100 μM)	0.35±0.05*	1.22±0.39*	2.44±0.59**

Specific (alaproclate-sensitive) transport of 5-HT was determined at 50, 100 and 500 nM [^{3}H]5-HT in the absence or presence of 100 μM 4-MTA, 4-MTMA or MDMA for 4 min at 37 °C. The results are the means±S.E.M. of at least three independent determinations measured in triplicate.

* $P<0.05$ compared to uptake of the corresponding concentration of [^{3}H]5-HT in the absence of drug.

** $P<0.01$ compared to uptake of the corresponding concentration of [^{3}H]5-HT in the absence of drug.

*** $P<0.001$ compared to uptake of the corresponding concentration of [^{3}H]5-HT in the absence of drug.

(2000). Briefly, rat whole brain was homogenised in ice-cold 10% sucrose containing 1 μM reserpine, 100 nM nomifensine and 100 nM GBR12935 to block any potential [^{3}H]5-HT reuptake into noradrenergic and dopaminergic nerve terminals. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C, and the supernatant ('synaptosomal preparation') was incubated with 5 nM [^{3}H]5-HT (specific activity 9.65×10^5 Bq (26 μCi)/nmol) in Krebs' phosphate buffer pH 7.4, containing 154.4 mM NaCl, 2.9 mM KCl, 4.9 mM KH_2PO_4, 1.1 mM $CaCl_2$, 0.83 mM $MgCl_2$, 5 mM glucose, 1 mg/ml ascorbic acid, 50 μM pargyline, 1 μM reserpine, 100 nM nomifensine and 100 nM GBR12935. After preincubation, synaptosomes (900 μl) were incubated for 5 min at 25 °C in the presence of test drug. The releasing action of each drug was terminated by dilution with 4 ml wash buffer (10 mM Tris–HCl, pH 7.4, containing 0.9% NaCl at 25 °C), followed by rapid vacuum filtration over GF/B filters. The quantity of radioactivity retained by the filters was determined by liquid scintillation counting following overnight solubilisation in 5 ml Ecoscint (National Diagnostics, UK). Nonspecific binding of [^{3}H]5-HT was measured by incubations in the presence of 100 μM tyramine. The results are expressed as the fraction (%) of radioactivity retained by the tissue in the absence of drug.

2.5. Measurement of aortic contraction

Descending thoracic aortae were isolated, mounted in a tissue bath, attached to a force transducer and maintained at 37 °C in Krebs'–Hensleit bicarbonate buffer of the following composition (mM): NaCl 112.6, KCl 4.7, KH_2PO_4 1.2, $MgSO_4-7H_2O$ 1.2, $NaHCO_3$ 25, (D)-(+)-glucose 12, $CaCl_2-2H_2O$ 1.9, gassed with 95% O_2/5% CO_2. After equilibration of rings for 45 min at a basal tension of 1.3 g, changes in isometric tension were measured after 15 min preincubation with drug, using the PowerLab data acquisition package.

2.6. Materials

[^{3}H]-5-hydroxytryptamine (specific activity=944 GBq (25.5 Ci)/mmol) was purchased from NEN, Amsterdam. Alaproclate HCl and nomifensine were purchased from RBI, UK. All other chemicals and compounds were purchased from Sigma, Dorset, UK and were of the highest grade commercially available.

2.7. Data analysis

Data were analysed using the software package KaleidaGraph under nonlinear regression analysis and fitting to either a one site rectangular hyperbola (Michaelis Menten curve) or sigmoidal concentration–response curve as appropriate. Determination of statistical significance was performed by a two-tailed unpaired Student's *t*-test, and a *P* value <0.05 was considered significant. Data are presented as the means±S.E.M. for *n* determinations as indicated.

3. Results

3.1. The effects of 4-MTA, 4-MTMA and MDMA on synaptosomal [^{3}H]5-HT transport

The maximum rate of [^{3}H]5-HT reuptake into isolated rat synaptosomes (V_{max}) was 20.16±2.77 pmol/min/mg protein with a K_m (substrate concentration required for half-maximal rate of uptake) of 395±99 nM. Addition of either 4-MTA, 4-MTMA or MDMA (100 μM) almost completely abolished [^{3}H]5-HT uptake over the entire concentration range of [^{3}H]5-HT (1–500 nM; Table 1). For example, at 500 nM [^{3}H]5-HT, 4-MTA, 4-MTMA, or MDMA reduced the rate of reuptake to 14%, 10%, or 13% of control, respectively. Kinetic analysis of [^{3}H]5-HT uptake in the presence of each of the drugs showed a significant reduction in the V_{max}, without any significant change in the K_m, as

Table 2
Kinetic analysis of synaptosomal [^{3}H]5-HT uptake by MDMA, 4-MTA and 4-MTMA

Drug	K_m (nM)	V_{max} (pmol/mg protein/min)
None	395 ± 99	20.16 ± 2.77
4-MTA	426 ± 106	4.95 ± 1.69*
4-MTMA	296 ± 120	3.66 ± 2.35*
MDMA	681 ± 155	6.11 ± 1.00**

Specific (alaproclate-sensitive) uptake was determined over a concentration range of 25–500 nM [^{3}H]5-HT for 4 min at 37 °C. Drugs (100 μM) were added at time zero of the assay, and the rate of uptake was determined at each concentration of substrate in triplicate in at least four independent experiments. The K_m and V_{max} were determined by nonlinear regression analysis by applying the Michaelis Menten equation to the data and are expressed as the means±S.E.M.

* $P<0.01$ compared to data obtained in the absence of drug.

** $P<0.05$ compared to data obtained in the absence of drug.

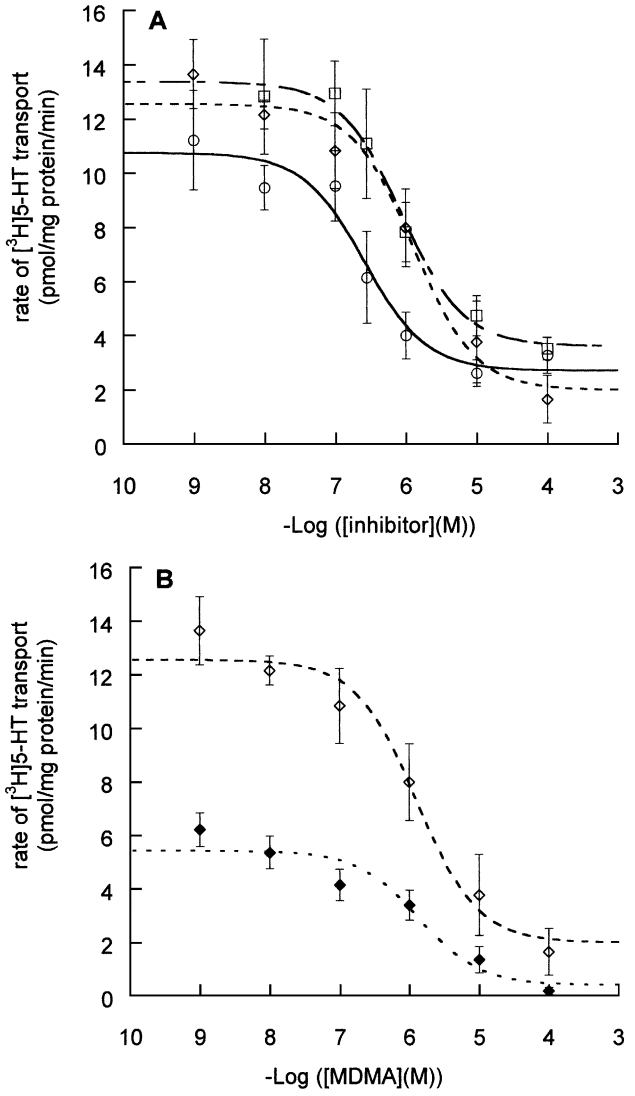

Fig. 2. Inhibition of synaptosomal 5-HT uptake by 4-MTA, 4-MTMA and MDMA. Synaptosomes were incubated for 4 min at 37 °C in the presence of [^{3}H]5-HT as specified in Section 2. (A) IC_{50} plot showing the inhibition of uptake of 500 nM [^{3}H]5-HT by 1 nM–1 mM 4-MTA (○), 4-MTMA (□) or MDMA (◇). (B) IC_{50} plot showing the inhibition of uptake of 50 nM (◆) and 500 nM (◇) [^{3}H]5-HT by MDMA. Data are presented as the means±S.E.M. of four individual experiments, each performed in triplicate. The data were analysed by fitting to a sigmoidal concentration–response curve using the KaleidaGraph application from Synergy®.

indicated in Table 2. To determine the IC_{50} for inhibition of [^{3}H]5-HT reuptake, a range of inhibitor concentrations (10 nM–100 μM) was tested at a substrate concentration of 500 nM 5-HT and the concentration–response curves for 4-MTA, 4-MTMA and MDMA are shown in Fig. 2A. The corresponding IC_{50} values (midpoints of each curve) were: 0.27 μM for 4-MTA (P<0.005 w.r.t. 4-MTMA or MDMA); 0.89 μM for 4-MTMA and 1.28 μM for MDMA. The IC_{50} for inhibition of [^{3}H]5-HT uptake by MDMA was not altered when the substrate concentration was lowered to 50 nM 5-HT (1.35 μM; Fig. 2B).

3.2. The effects of 4-MTA, 4-MTMA and MDMA on [^{3}H]5-HT release from synaptosomes

4-MTA, 4-MTMA and MDMA (1–100 μM) all caused release of preloaded [^{3}H]5-HT from synaptosomes, but the quantity of [^{3}H]5-HT released by MDMA was significantly greater than for either 4-MTA or 4-MTMA. For example, the quantity of radioactivity retained by the synaptosomes after 5 min incubation with 10 μM MDMA fell to 42.7±3.5% of control, whereas this figure was 60.0±2.7% of control for 10 μM 4-MTA and 58.3±4.3% of control for 10 μM 4-MTMA (Fig. 3). With 100 μM drug, the quantity of [^{3}H]5-HT released was 52.0±2.5% (MDMA), 70.3±2.3% (4-MTA) and 66.5±4.6% of control (4-MTMA). There was no significant difference in the amount of [^{3}H]5-HT released by 4-MTA and 4-MTMA at any of the concentrations tested.

3.3. The effects of 4-MTA, 4-MTMA and MDMA on 5-HT-induced rat aortic ring contraction

Preincubation of aortic rings with 100 μM 4-MTA for 15 min significantly reduced (P<0.05) the maximal contraction (E_{max}) to 5-HT to 71% (0.84±0.03 g) that of control rings (1.20±0.06 g), as shown in Fig. 4A. Similar treatment with 4-MTMA significantly reduced (P<0.05) the E_{max} to 91% (1.07±0.05 g) that of control rings (1.17±0.07 g; Fig. 4B) while control E_{max} values (0.92±0.02 g) were not significantly altered (0.82±0.02 g) in rings treated with 100 μM MDMA (Fig. 4C). The concentrations of 5-HT required for half-maximal contraction (EC_{50}) in control, 4-MTA-treated and 4-MTMA-treated tissues were 4.13, 8.75 and 20.08 μM,

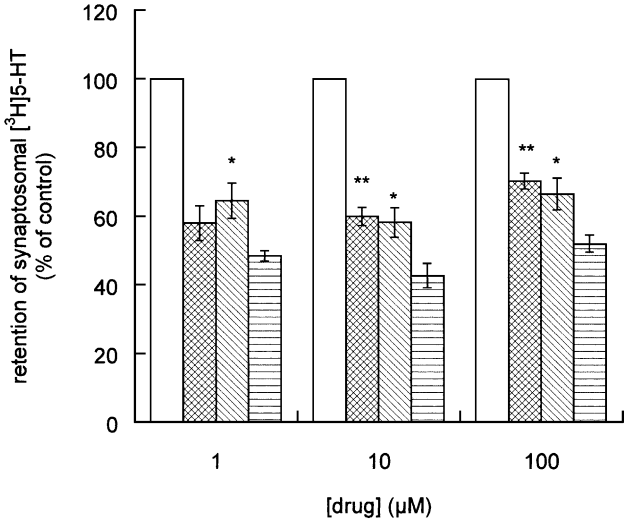

Fig. 3. Stimulation of efflux of [^{3}H]5-HT from synaptosomes by 4-MTA, 4-MTMA and MDMA. Release of preloaded [^{3}H]5-HT was measured for 5 min at 25 °C in the presence of 1, 10 and 100 μM 4-MTA, 4-MTMA or MDMA, as described in Section 2. The results are expressed as the fraction (%) of the radioactivity retained by the tissue in the absence of drug, and are presented as the means±S.E.M. of four separate experiments, each measured in triplicate. □ No drug, ▨ 4-MTA, ▧ 4-MTMA, ▤ MDMA.

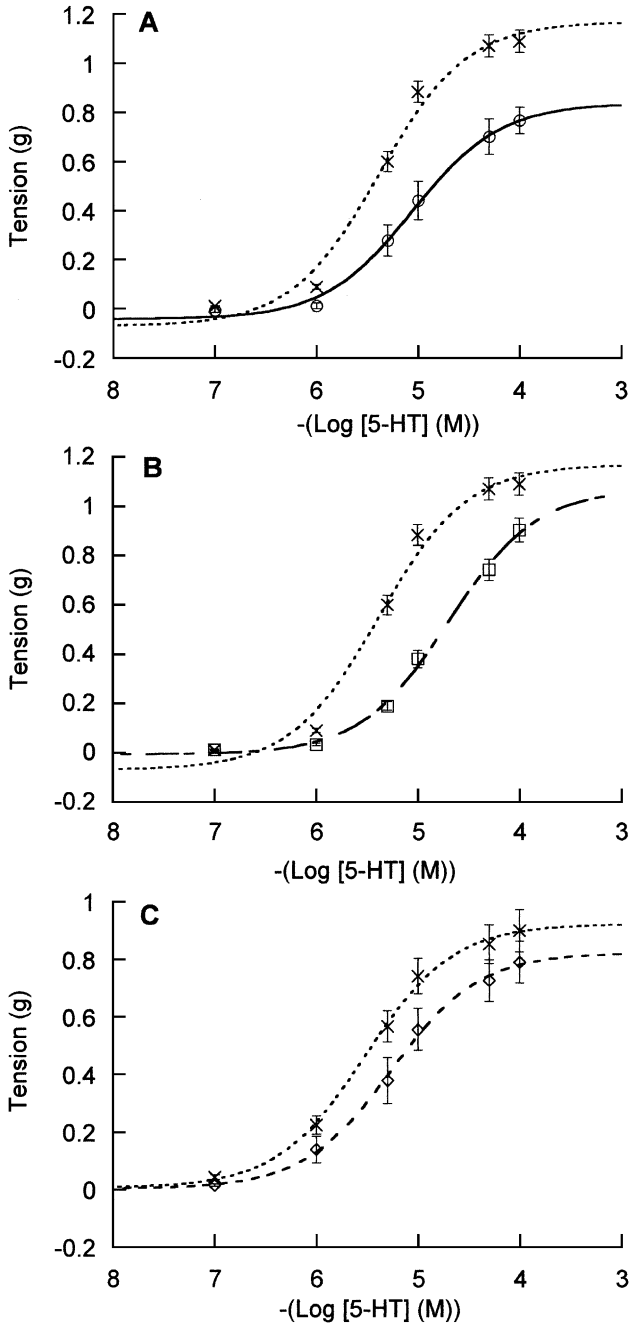

Fig. 4. The effect of 4-MTA, 4-MTMA and MDMA on 5-HT induced contraction in the rat thoracic aortic ring. Isolated rings were mounted in a tissue bath, and either 100 μM 4-MTA (○) (A), 4-MTMA (□) (B) or MDMA (◇) (C) were added 15 min prior to addition of 5-HT. Control responses to 5-HT are also shown (×). Data are presented as the means±S.E.M. of five (4-MTA, 4-MTMA) or nine (MDMA) individual experiments, each performed in duplicate. The data were analysed by fitting to a sigmoidal concentration–response curve to determine the EC_{50} and E_{max} for aortic contraction.

respectively (EC_{50} for 4-MTMA significantly different from that for control, and 4-MTA, $P<0.0001$ in each case). In separate experiments, EC_{50} values for control and MDMA-treated tissues were not significantly different (2.99 and 5.39 μM, respectively). Additionally, the EC_{50} in the presence of 4-MTA was not significantly different from control. Neither 4-MTA, 4-MTMA, nor MDMA themselves induced contraction in the aorta in the absence of 5-HT (data not shown).

4. Discussion

This study shows that 4-MTA and 4-MTMA, like MDMA target 5-HT signalling systems in brain and vasculature. In the brain, MDMA, 4-MTA and 4-MTMA are all effective inhibitors of synaptosomal [^{3}H]5-HT transport (Fig. 1). This is manifest as micromolar IC_{50} values for [^{3}H]5-HT reuptake into cerebral synaptosomes. Steele et al. (1987) also showed that synaptosomal uptake of 5-HT is directly blocked by MDMA, with an IC_{50} that compares well with the data from our experiments. In the case of 4-MTA, our data confirm the earlier work of Huang et al. (1992) and show that 4-MTA is more potent than MDMA. In a separate study reported by Johnson et al. (1991), another para-substituted amphetamine, *p*-chloroamphetamine was also more potent than MDMA. N-methylation, however, appears to result in a reduction of inhibitory potency, since 4-MTMA and MDMA were both less effective inhibitors of [^{3}H]5-HT reuptake than the nonmethylated analogue, 4-MTA. Stone et al. (1987) compared the neurotoxic potential of 3,4-methylenedioxyamphetamine (MDA) with that of its N-methylated (MDMA) and N-ethylated derivatives: in that case, both MDA and MDMA dramatically decreased brain concentrations of 5-HT, whereas the N-ethylated compound was much less potent. The present findings strengthen the conclusions of Stone et al. (1987) that increased N-alkyl substitution leads to reduced potency.

Experiments have shown that both 4-MTA and 4-MTMA cause release of preloaded [^{3}H]5-HT from synaptosomes, but that at the concentrations tested, they are less effective in this regard than MDMA. Huang et al. (1992) also demonstrated that 4-MTA stimulated [^{3}H]5-HT release from rat cortical slices over a similar concentration range and with a potency comparable to that of *p*-chloroamphetamine. The greater releasing action of MDMA shown in our experiments suggests that replacement of the methylenedioxy group in MDMA with the methylthio moiety in 4-MTA and 4-MTMA has a greater impact on the ability of these compounds to cause release of 5-HT than does methylation of the side chain, as in MDMA and 4-MTMA. Thus, the structural specificity for stimulation of 5-HT release differs from that of either inhibition of 5-HT transport or 5-HT-mediated contraction of the aorta, which is discussed below.

There is no direct evidence demonstrating accumulation of MDMA, 4-MTA or 4-MTMA into synaptosomes, nevertheless, the ability of these compounds to stimulate 5-HT release is consistent with their being taken up by the 5-HT transporter, resulting in drug/5-HT exchange, which would not be expected for a nontransported inhibitor. This view is strengthened by the observation that the 5-HT releasing action of MDMA can be blocked by 5-HT uptake inhibitors,

such as fluoxetine (Hekmatpanah and Peroutka, 1990), and the same is true for *p*-chloroamphetamine-stimulated 5-HT release (Rudnick and Wall, 1992). It is likely that 4-MTA and 4-MTMA also act by a similar mechanism, although our data do not rule out the possibility that these compounds, by binding to the transporter, initiate uncoupled efflux of 5-HT.

The altered order of potency of inhibition of transport between 4-MTA, 4-MTMA and MDMA, as opposed to stimulation of 5-HT efflux, may be an indication that structural modification of MDMA alters the balance between behaviour as a substrate or as an inhibitor of the 5-HT transporter: 4-MTA and 4-MTMA may not be as well taken up as MDMA, although they are better inhibitors of 5-HT transport.

Both 4-MTA and 4-MTMA were effective inhibitors of 5-HT-mediated contraction of the rat aorta in vitro. This result is particularly interesting, in view of the fact that MDMA had no significant effect in this respect and raises the question of how 4-MTA and 4-MTMA might differ in their mechanism of action from MDMA. In contrast to the brain, MDMA does not inhibit transport of 5-HT by non-neuronal transporters in the aorta (Cannon et al., 2001). It is not known whether 4-MTA or 4-MTMA affect the function of such transporters in the peripheral vasculature, but an inhibition of 5-HT reuptake into nonneuronal tissue would not be expected to lead to the reduced 5-HT responsiveness observed in our experiments The fact that neither 4-MTA nor 4-MTMA altered contractility directly (data not shown), coupled with the observed shifts in the concentration–response curves (Fig. 4A and B) suggests that these agents may be acting as antagonists, though the nature of this antagonism is unclear at this stage.

Recent studies indicate that 5-HT-mediated rat aortic contraction occurs via 5-HT_{2A} receptors (Florian and Watts, 1998). It is possible, therefore, that 4-MTA and 4-MTMA exert their antagonist action due to an affinity for 5-HT_{2A} receptors. Other substituted amphetamines, including MDMA and MDA also have affinity for 5-HT_{2A} receptors, though as agonists: in a study reported by Nash et al. (1994), MDA had considerably greater efficacy in a cell line expressing 5-HT_{2A} receptors. It is intriguing to note that the greater degree of inhibition of 5-HT-induced contraction by 4-MTA, the N-demethylated analogue of 4-MTMA, mirrors the greater efficacy of MDA, the N-demethylated analogue of MDMA, at 5-HT_{2A} receptors (Nash et al., 1994). The neurotoxicity of high doses of MDA or MDMA may, however, be mediated by 5-HT_{2C} receptors, as proposed by Nash et al. (1994).

Inhibition of 5-HT-mediated responses in the aorta by 4-MTA and 4-MTMA raises the question of whether the peripheral action of these compounds may be associated with toxicity in humans. Available evidence suggests that in rats high (acute) doses of 4-MTA are fatal (EMCDDA, 1999), yet administration of 4-MTA does not result in neurotoxicity one week later (Huang et al., 1992). Thus, 4-MTA may exert an early action on peripheral vasculature, which is followed later by a central action; this might also explain the reportedly slower onset of its central effects compared to MDMA (EMCDDA, 1999).

The evidence presented here for peripheral vascular effects of 4-methylthio substituted amphetamines would be consistent with targeting by these agents of a wide range of peripheral organs, following oral administration. Indeed, significant levels of MDMA are known to be concentrated in organs such as the liver, bladder and intestine following oral administration (Cho et al., 1990; Sakai et al., 1983), and it is likely that the 4-methylthio compounds, being lipophilic, would also accumulate at these sites.

In conclusion, this study demonstrates that whereas 4-MTA is a more potent inhibitor than MDMA and 4-MTMA of [^{3}H]5-HT reuptake in the brain, MDMA is most potent at causing [^{3}H]5-HT release. Both 4-MTA and 4-MTMA, unlike MDMA, are potent inhibitors of 5-HT-mediated responses in the vasculature. Whether MDMA and its 4-methylthio analogues specifically target 5-HT systems, or also modulate noradrenergic neurotransmission in the brain and periphery, is currently the subject of a comprehensive evaluation of the central and peripheral vascular toxicity of these compounds.

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