Effects of the $\alpha_2$-adrenergic agonist clonidine on the pharmacodynamics and pharmacokinetics of methylenedioxymethamphetamine in healthy volunteers

Cédric M. Hysek, Robin Brugger, Linda D. Simmler, Marcel Bruggisser, Massimiliano Donzelli, Eric Grouzmann, Marius C. Hoener, Matthias E. Liechti

Division of Clinical Pharmacology and Toxicology, Department of Biomedicine and Department of Internal Medicine, University Hospital and University of Basel, Switzerland (C.M.H., R.B, L.D.S., M.B., M.D., M.E.L.); Division of Clinical Pharmacology and Toxicology, University Hospital, Lausanne, Switzerland (E.G.); Pharmaceuticals Division, Neuroscience Research, F. Hoffmann-La Roche Ltd., Basel, Switzerland (M.C.H.).
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Correspondence:
Dr. Matthias E Liechti, Division of Clinical Pharmacology and Toxicology, University Hospital Basel, Hebelstrasse 2, CH-4031 Basel, Switzerland; Tel: +41 61 328 68 68, Fax: +41 61 265 45 60,
E-mail: mliechti@uhbs.ch

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Abbreviations: AMRS, Adjective Mood Rating Scale; AUC, area under the plasma concentration-time curve; AUEC, area under the effect-time curve; C_{max}, maximal plasma concentration; CYP, cytochrome P450; DA, dopamine; 5D-ASC, 5-Dimensions of Altered States of Consciousness; E_{max}, maximal effect; EC_{50}, plasma concentration at which 50 % of the maximal effect is reached; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine (serotonin); LC, List of Complaints; MAP, mean arterial pressure; MDA, 3,4-methylenedioxyamphetamine; MDMA, ±3,4-methylenedioxymethamphetamine; NE, norepinephrine; PK, pharmacokinetic; PD, pharmacodynamic; t_{max}, time to maximal plasma concentration; STAI, State-Trait Anxiety Inventory; VAS, Visual Analog Scale.
Chemical compounds: Duloxetine-d7, C_{18}H_{12}D_{7}NOS; MDA, C_{10}H_{13}NO_{2}; MDA-d4, C_{10}H_{9}D_{4}NO_{2}; MDMA, C_{11}H_{13}NO_{2}; MDMA-d5, C_{11}H_{10}D_{5}NO_{2}; Methanol, CH_{3}O; Pholedrine, C_{10}H_{15}NO.

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Abstract

The mechanism of action of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) involves the carrier-mediated and potentially vesicular release of monoamines. We assessed the effects of the sympatholytic $\alpha_2$-adrenergic receptor agonist clonidine (150 $\mu$g, p.o.), which inhibits the neuronal vesicular release of norepinephrine, on the cardiovascular and psychotropic response to MDMA (125 mg, p.o.) in 16 healthy subjects. The study used a randomized, double-blind, placebo-controlled crossover design with four experimental sessions. The administration of clonidine 1 h before MDMA reduced the MDMA-induced increases in plasma norepinephrine concentrations and blood pressure but only to the extent that clonidine lowered norepinephrine levels and blood pressure compared with placebo. Thus, no interaction was found between the cardiovascular effects of the two drugs. Clonidine did not affect the psychotropic or pharmacokinetics of MDMA. The lack of an interaction of the effects of clonidine and MDMA indicates that vesicular release of norepinephrine, which is inhibited by clonidine, does not critically contribute to the effects of MDMA in humans. Although clonidine may be used in the treatment of stimulant-induced hypertensive reactions, the present findings do not support a role for $\alpha_2$-adrenergic receptor agonists in the prevention of psychostimulant dependence.
Introduction

The sympathomimetic amphetamine derivative 3,4-methylenedioxyamphetamine (MDMA, ecstasy) releases norepinephrine (NE), serotonin (5-hydroxytryptamine [5-HT]), and dopamine (DA) from nerve terminals via their corresponding presynaptic monoamine transporters (Rudnick and Wall, 1992; Rothman et al., 2001; Verrico et al., 2007). The psychotropic and cardiostimulant effects of MDMA in humans appear to depend on the transporter-mediated release of NE and 5-HT. Both the subjective and cardiovascular responses to MDMA can be reduced by blocking the NE or 5-HT transporter using selective transporter inhibitors (Liechti et al., 2000; Farre et al., 2007; Tancer and Johanson, 2007; Hysek et al., 2011). The release of NE has been shown to critically mediate the effects of psychostimulants (Rothman et al., 2001; Sofuoglu et al., 2009), including MDMA (Hysek et al., 2011; Newton, 2011). However, MDMA-induced increases in extracellular monoamines may also result from impulse-dependent vesicular/exocytotic release or transmitter uptake inhibition (Seiden et al., 1993; Florin et al., 1994; Hondebrink et al., 2011). The vesicular release of NE is under negative feedback control mediated by presynaptic \(\alpha_2\)-adrenergic receptors (Buccafusco, 1992; Starke, 2001), and \(\alpha_2\) receptors are thereby involved in noradrenergic function, including vascular contraction, blood pressure control, body temperature regulation, arousal, and memory (Starke, 2001). Clonidine is an \(\alpha_2\)-adrenergic receptor agonist and sympatholytic drug that reduces noradrenergic activity by decreasing the impulse-mediated vesicular release of NE (Buccafusco, 1992; Philipp et al., 2002). In healthy subjects, clonidine dose-dependently suppressed plasma levels of NE (Veith et al., 1984), blood pressure (Mitchell et al., 2005), and cardiac output, lowered body temperature (Bexis and Docherty, 2005), and had sedative effects (Hall et al., 2001). These sympatholytic effects of clonidine are opposite to the clinical effects of sympathomimetic drugs, including MDMA. Therefore, clonidine has been recommended in the treatment of MDMA intoxication.
(Green et al., 1995; Liechti, 2003), and it is routinely used to control sympathetic activation during withdrawal from drugs of abuse.

In rats, clonidine blocked the behavioral response and hippocampal and prefrontal NE release after treatment with cocaine or low doses of amphetamine (Florin et al., 1994; Carey et al., 2008). Clonidine may therefore reduce stimulant-induced NE release and the associated behavioral effects of psychostimulants and may even be used in the treatment of stimulant addiction. In fact, clonidine prevented amphetamine-induced psychomotor stimulation (Vanderschuren et al., 2003) and cue-induced cocaine seeking in rats (Smith and Aston-Jones, 2011) and drug craving in cocaine users (Jobes et al., 2011).

The effects of clonidine on the acute response to psychostimulants have not yet been evaluated in humans. In the present study, we assessed the interactive pharmacodynamic effects of clonidine and MDMA in healthy subjects. We expected that clonidine would reduce the effects of MDMA in humans to the extent that the clinical effects of MDMA are mediated by the exocytotic release of NE.
Materials and Methods

Study design

We used a double-blind, placebo-controlled, randomized, crossover design with four experiential conditions (placebo-placebo, clonidine-placebo, placebo-MDMA, and clonidine-MDMA) in a balanced order. The washout periods between sessions were 10-14 days long. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines on Good Clinical Practice and approved by the Ethics Committee of the Canton of Basel, Switzerland. The use of MDMA in healthy subjects was authorized by the Swiss Federal Office of Public Health, Bern, Switzerland. The study was registered at ClinicalTrials.gov (NCT01136278).

Study procedures

The subjects completed a screening session, four test sessions, and an end-of-study visit. The test sessions were conducted in a quiet hospital research ward with no more than two research subjects present per session. Prior to undergoing the test sessions, the subjects were asked about potential health problems. Drug tests and urine tests to determine pregnancy were also performed. An indwelling intravenous catheter was placed in the antecubital vein for blood sampling. Clonidine (150 μg, p.o.) or placebo was administered at 8:00 AM. MDMA (125 mg, p.o.) or placebo was administered at 9:00 PM. A standardized lunch was served at 12:00 PM, and the subjects were sent home at 3:00 PM. Outcome measures were assessed repeatedly before and after drug administration.

Subjects

Sixteen healthy subjects (eight men, eight women) with a mean ± SD age of 25.4 ± 4.9 years were recruited on the university campus. The exclusion criteria included the following; (i) age < 18 or > 45 years, pregnancy determined by a urine test before each test session, (ii)
body mass index < 18.5 kg/m² or > 25 kg/m², (iii) personal or family (first-degree relative) history of psychiatric disorder (determined by the structured clinical interview for Axis I and Axis II disorders according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (Wittchen et al., 1997) supplemented by the SCL-90-R Symptom Checklist (Derogatis et al., 1976; Schmitz et al., 2000), Freiburg Personality Inventory (Fahrenberg et al., 1984), and Trait Scale of the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970), (iv) the regular use of medications, (v) chronic or acute physical illness assessed by physical examination, electrocardiogram, standard hematology, and chemical blood analyses, (vi) smoking more than 10 cigarettes per day, (vii) a lifetime history of using illicit drugs more than five times, with the exception of cannabis), (viii) illicit drug use within the last 2 months, and (ix) illicit drug use during the study determined by urine tests conducted before the test sessions using TRIAGE 8 (Biosite, San Diego, CA). The subjects were asked to abstain from excessive alcohol consumption between test sessions and limit alcohol use to one glass on the day before each test session. All of the subjects were non-smokers. Twelve subjects had previously used cannabis. Five subjects reported using illicit drugs once, in which one subject had tried lysergic acid diethylamide, ecstasy, and psilocybin, two had tried cocaine and psilocybin, one had tried ecstasy, and one had tried psilocybin. All of the subjects were phenotyped for cytochrome P450 (CYP) 2D6 activity using dextromethorphan as the probe drug. Eight extensive, seven intermediate, and one poor CYP2D6 metabolizer were identified in the study. The female subjects were investigated during the follicular phase (day 2-14) of their menstrual cycle when the reactivity to amphetamines is expected to be similar to men (White et al., 2002). All of the subjects provided their written informed consent before participating in the study, and they were paid for their participation.

*Drugs*

(±) MDMA hydrochloride (C₁₁H₁₅NO₂, Lipomed AG, Arlesheim, Switzerland) was obtained from the Swiss Federal Office of Public Health and prepared as gelatin capsules
(100 and 25 mg). Identical placebo (lactose) capsules were prepared. MDMA was administered in a single absolute oral dose of 125 mg, corresponding to a dose of $1.88 \pm 0.28$ mg/kg body weight. Clonidine tablets (150 \(\mu\)g, Catapresan, Boehringer Ingelheim GmbH, Basel, Switzerland) were encapsulated within opaque gelatin capsules, and identical placebo (lactose) capsules were prepared. Clonidine (150 \(\mu\)g) or placebo was administered 1 h before MDMA (125 mg) or placebo administration. Oral medication administration was supervised by study personnel.

**Pharmacodynamic measurements**

Psychometric scales. Subjective measures were assessed using Visual Analog Scales (VAS) (Hysek et al., 2011), the Adjective Mood Rating Scale (AMRS) (Janke and Debus, 1978), the 5-Dimensions of Altered States of Consciousness (5D-ASC) (Dittrich, 1998; Studerus et al., 2010), and the STAI (Spielberger et al., 1970). VASs included “any drug effect”, “good drug effect”, “bad drug effect”, “drug liking”, “drug high”, “stimulated”, “tiredness”, “closeness to others”, and “open” (Farre et al., 2007; Tancer and Johanson, 2007; Kolbrich et al., 2008; Hysek et al., 2011). The VASs were presented as 100 mm horizontal lines marked “not at all” on the left and “extremely” on the right. The VAS for “closeness to others”, and “open” were bidirectional (± 50 mm). The VASs were administered 1 h before and 0, 0.33, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 5 h after MDMA or placebo administration. The 60-item Likert-type scale short version of the AMRS (Janke and Debus, 1978) was administered 1 h before and 1.25, 2, and 5 h after MDMA or placebo administration. The AMRS contains subscales for activity, inactivation, extroversion and introversion, well-being, emotional excitation, anxiety-depression, and dreaminess. The 5D-ASC rating scale measures alterations in mood, perception, experience of self in relation to environment, and thought disorder (Studerus et al., 2010). The 5D-ASC rating scale comprises five subscales or dimensions (Dittrich, 1998) and 11 lower-order scales (Studerus et al., 2010). The 5D-ASC dimension “oceanic boundlessness” (27 items) measures...
derealization and depersonalization associated with positive emotional states ranging from heightened mood to euphoric exaltation. The dimension “anxious ego dissolution” (21 items) summarizes ego disintegration and loss of self-control, two phenomena associated with anxiety. The corresponding lower-order scales included: “disembodiment”, “impaired control of cognition”, and “anxiety”. The dimension “visionary restructuralization” (18 items) consists of the lower-order scales “complex imagery”, “elementary imagery”, “audiovisual synaesthesia”, and “changed meaning of percepts”. The dimension “auditory alterations” (16 items) subsumes auditory (pseudo) hallucinations, and the dimension “vigilance reduction” (12 items) describes states of drowsiness and impaired alertness and cognitive performance. The global ASC score was determined by adding the “oceanic boundlessness”, “anxious ego dissolution”, and “visionary restructuralization” scores. The 5D-ASC scale was administered 4 h after MDMA or placebo administration. The STAI state-anxiety subscale (Spielberger et al., 1970) was administered 1 h before and 1.25, 2, and 5 h after MDMA or placebo administration. Physiologic measures. Physiologic measures were assessed repeatedly 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. Heart rate, systolic blood pressure, and diastolic blood pressure were measured using an OMRON M7 blood pressure monitor (OMRON Healthcare Europe, Hoofddorp, The Netherlands) in the dominant arm after a resting time of 5 min. Measures were taken twice per time point with an interval of 1 min, and the average was used for analysis. Core (tympanic) temperature was assessed using a GENIUS 2 ear thermometer (Tyco Healthcare Group, Watertown, NY).

Adverse effects

Adverse effects were assessed 1 h before and 3 and 24 h after MDMA or placebo administration using the List of Complaints (LC; Zerssen, 1976; Hysek et al., 2011). The scale consists of 66 items that yield a total adverse effects score, reliably measuring physical and general discomfort.
Laboratory analyses

Samples of whole blood for the determination of MDMA and 3,4-methylenedioxyamphetamine (MDA), the active metabolite of MDMA, were collected 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, and 6 h after MDMA or placebo administration. Blood samples to determine concentrations of NE and epinephrine were taken at 1 h before, and 1 and 2 h after MDMA or placebo administration. All blood samples were collected on ice and centrifuged within 10 min at 4 ºC. The plasma was then stored at -20ºC until analysis. The plasma levels of free catecholamines (NE and epinephrine) were determined by high-performance liquid chromatography (HPLC) with an electrochemical detector as described previously (Hysek et al., 2011). The plasma concentrations of MDMA and MDA were determined using HPLC coupled to tandem mass spectrometry. The analytes were extracted by protein precipitation using methanol that contained 0.1 μg/ml MDMA-d5, MDA-d4 (both from Lipomed, Arlesheim, Switzerland), duloxetine-d7 (Toronto Research Chemicals, Canada), and pholedrine (Sigma, Buchs, Switzerland). Chromatographic separation was performed on a Shimadzu HPLC system that consisted of a HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland), two Shimadzu LC-20 AD pumps (Shimadzu, Reinach, Switzerland) controlled by a Shimadzu CBM-20A unit, a Shimadzu CTO-20AD column oven and a six port VICI valve (VICI, Schenkon, Switzerland). A Chromolith SpeedROD RP-18e column (50 × 4.6 mm, WVR Dietikon, Switzerland) was used for the separation of the analytes. Eluent A (0.1% formic acid in water) and eluent B (0.1% formic acid in methanol) were used in the following gradient: 100% A for 0-1 min, 20-95% B for 1-4 min, 95% B for 4-5 min, and 100% A for 5-6 min. The mobile phases were delivered at a constant flow rate of 0.8 ml/min. The total run time was 6.0 min. The column oven was set at 35ºC. The injection volume was 10 µl. Mass spectrometric detection was performed using a triple quadrupole mass spectrometer (API4000, Applied Biosystems, Rotkreuz, Switzerland) operated in electrospray-ionization positive-ion mode. The samples were quantified using peak area
ratios. The assays were linear in the concentration range of 1-1000 ng/ml for MDMA and MDA. The performance of the method was monitored using quality control samples at the lower limit of quantification and at two or three concentrations. The inter-assay accuracy for the quality control-samples ranged from 97.5% to 100% for MDMA and from 95.3% to 103% for MDA. Interassay precision values ranged from 2.8% to 8.0% for MDMA and from 3.8% to 10.5% for MDA.

**Pharmacokinetics and pharmacokinetic-pharmacodynamic (PK-PD) modeling**

Pharmacokinetics. The data for the plasma concentrations of MDMA and MDA were analyzed using non-compartmental methods. Maximal plasma concentration (C_max) and time to C_max (t_max) were obtained directly from the concentration-time curves of the observed values. The terminal elimination rate constant (λ_z) for MDMA was estimated by log-linear regression after semilogarithmic transformation of the data, using the last two to three data points of the terminal linear phase of the concentration-time curve of MDMA. The terminal elimination half-life (t_1/2) was calculated using λ_z and the equation t_1/2 = ln2/λ_z. The area under the plasma concentration-time curve (AUC)_{0-6 h} was calculated using the linear trapezoidal rule. Plasma concentrations were only determined up to 6 h after MDMA administration because the aim of the study was to assess potential changes in plasma levels of MDMA during the time of the pharmacodynamic effects of MDMA. Determining the t_1/2 for MDA was not possible because of its long t_1/2 which would require an extended sampling time.

PK-PD modeling. We evaluated the in vivo relationship between the MDMA concentration and the effect of MDMA on mean arterial pressure (MAP) using a soft-link PK-PD model (Meibohm and Derendorf, 1997). Blood pressure and plasma concentrations were assessed at the same time-points. Because we observed clockwise hysteresis in the effect-concentration relationship over time, we used PK-PD data pairs within the ascending part of the individual curves up to the maximal effect (E_{max}) or C_max. Our estimation of E_{max}, which should represent the maximal response portion of the dose-response curve, may already
have been affected by acute tolerance. However, $E_{\text{max}}$ values of 100% (scale maximum) or stable high values were reached by most subjects despite possible tolerance. Based on the good brain penetration of MDMA and absence of a time lag, we assumed rapid equilibration between the plasma and central compartment (brain). A sigmoidal dose-response (variable slope) model was fitted to the pooled data of all individuals: 

$$E = \frac{E_{\text{max}}}{1 + 10^{(\log EC_{50} - C_P) \times h}}$$

in which $E$ is the observed effect, $C_P$ is the plasma MDMA concentration, $EC_{50}$ is the plasma concentration at which 50% of the maximal effect is reached, $E_{\text{max}}$ is the maximal effect, and $h$ is the Hill slope. The sigmoidal dose-response model provided the best fit to the data and a better fit than a simple $E_{\text{max}}$ or linear model. Data pooling was used because only few data pairs were available for each subject. Non-linear regression was used to obtain parameter estimates.

**Statistical analyses**

Values were transformed to differences from baseline. The $E_{\text{max}}$ and AUEC values were determined for repeated measures and compared by one-way General Linear Models repeated-measures analysis of variance (ANOVA) with drug treatment as a factor, using STATISTICA 6.0 software (StatSoft, Tulsa, OK, USA). Tukey post hoc comparisons were performed based on significant main effects of treatment. Additional two-way ANOVAs, with the two drug factors MDMA (MDMA vs. placebo) and clonidine (clonidine vs. placebo) were used to test for interactive vs. additive effects of the two drugs on physiological measures or blood levels of catecholamines. Additional ANOVAs were performed, with drug order as an additional factor, to exclude carryover effects. The criterion for significance was $p < 0.05$.

Mean arterial pressure was calculated from diastolic blood pressure and systolic blood pressure using the formula $\text{MAP} = DBP + (SBP-DBP)/3$. 

Results

**Neuroendocrine and cardiovascular effects**

MDMA increased the level of circulating NE, an endocrine marker of sympathetic nervous system activation, and elevated blood pressure and heart rate compared with placebo (Fig. 1b and 2a and b, Table 1). Clonidine prevented the MDMA-induced increase in plasma NE (Fig. 1b, Table 1). It also attenuated the blood pressure response to MDMA, although reflected only by the AUEC and not $E_{\text{max}}$ (Fig. 2a, Table 1). Clonidine also decreased the level of circulating NE and blood pressure compared with placebo to a similar extent as the reduction in NE and pressure elevations induced by MDMA (Fig. 1b and 2a-c, Table 1). Additional ANOVAs with two drug factors, MDMA and clonidine, yielded significant main effects of MDMA and clonidine on $E_{\text{max}}$ values of MAP ($F_{1,15} = 106.1$ and $18.1$, respectively, both $p < 0.001$) but no MDMA × clonidine interaction ($F_{1,15} = 0.2$, $p = 0.7$), consistent with an additive effect of the two drugs. Similarly, the ANOVA of NE levels showed significant main effects of MDMA and clonidine ($F_{1,15} = 41.5$ and $34.2$, respectively, both $p < 0.001$) but no MDMA × clonidine interaction ($F_{1,15} = 0.0$, $p = 1$). The circulating levels of epinephrine were not significantly altered by the drugs and clonidine did not affect the increase in heart rate produced by MDMA (Fig. 2b, Table 1).

**Psychotropic effects**

Overall, clonidine had no effect on the psychotropic response to MDMA. It did not significantly affect the MDMA-induced increases in VAS ratings of subjective effects or AMRS scores (Fig. 3 and 4, Table 1), although it weakly but nonsignificantly attenuated “good drug effect”, “drug liking”, and “drug high” produced by MDMA (Fig. 3). Clonidine alone increased the VAS score for “tiredness” and AMRS score for “inactivation” compared with placebo (Fig. 3 and 4, Table 1). Clonidine had no effect on the robust changes produced by MDMA on the 5D-ASC rating scale (Fig. 5). Neither clonidine nor MDMA altered the state anxiety scale scores on the STAI (Table 1).

**Adverse effects**
MDMA increased the total adverse effects score on the LC both 3 and 24 h after administration compared with placebo (Table 1). The adverse effects of clonidine and MDMA were additive (Table 1). Thus, clonidine did not affect the untoward effects of MDMA. The frequently reported adverse effects of MDMA included a lack of appetite (n = 11), restlessness (n = 11), thirst (n = 10), sweating (n = 8), and bruxism (n = 8). Tiredness was typically reported after administration of clonidine-placebo and clonidine-MDMA (n = 11 and 10, respectively). No severe adverse effects were reported.

**Pharmacokinetics and PK-PD relationship**

Clonidine did not affect the plasma concentration-time curves of MDMA or MDA (Fig. 6a and b, Table 2). The pharmacokinetic parameters of MDMA were not dependent on CYP2D6 phenotype. However, the sample was small, and only one subject was a poor metabolizer. Fig. 6c and d show the effects of MDMA on blood pressure in terms of plasma concentration. The hysteresis loop shows that the MDMA-induced changes in MAP returned to baseline within 6 h when MDMA concentrations were still high (clockwise hysteresis), consistent with acute pharmacodynamic tolerance (Fig. 6c). Clonidine reduced the MDMA-induced blood pressure response for all MDMA plasma concentrations reflected by a downward shift in the concentration-pressure effect curve of MDMA (Fig. 6c). This shift was similar to the blood pressure-lowering effect of clonidine alone compared with placebo. Thus, the effects of the drugs were additive. Clonidine did not affect the EC_{50} value of the concentration-pressure effect curve of MDMA (Fig. 6d).
Discussion

In the present study, the α2-adrenergic receptor agonist clonidine reduced the elevations in the plasma concentration of NE and increases in blood pressure in response to MDMA. However, clonidine decreased plasma NE levels and blood pressure to a similar extent as the decreases in the response to MDMA when its effects were compared with placebo. Thus, the sympatholytic effects of clonidine and the sympathomimetic effects of MDMA were additive with no interaction between the effects of MDMA and clonidine on the noradrenergic system. Additionally, clonidine did not affect the psychotropic effects of MDMA, although clonidine was used in this study in a relatively high single dose that produced sympatholytic effects, including lower plasma NE levels, decreased blood pressure, and sedation on all psychometric scales compared with placebo, findings consistent with previous studies (Keranen et al., 1978; Anavekar et al., 1982).

We used the sympatholytic drug clonidine to block the MDMA-induced impulse-dependent vesicular release of NE (Florin et al., 1994). The fact that clonidine did not interact with the clinical effects of MDMA in the present study indicates that the vesicular release of NE is not involved in the mediation of the effects of MDMA in humans. The finding indirectly supports the view that the effects of MDMA in humans primarily depend on the transporter-mediated release of NE, 5-HT, and possibly DA (Liechti et al., 2000; Hysek et al., 2011). A preclinical study that used microdialysis in rats showed that clonidine reduced the NE response only to a low dose of amphetamine, but clonidine became less effective as the dose of amphetamine increased (Florin et al., 1994). This result suggests that amphetamine acts primarily as a transporter-mediated NE releaser at higher doses (Florin et al., 1994). In contrast to our study, clonidine prevented behavior relevant to stimulant addiction, including amphetamine-induced psychomotor stimulation (Vanderschuren et al., 2003) and cue-induced cocaine seeking in rats (Smith and Aston-Jones, 2011) and drug craving in cocaine users (Jobes et al., 2011). Altogether, the previous preclinical studies and our clinical
findings indicate that amphetamines, including MDMA, do not increase NE impulse flow and that their action in humans depends on transporter-mediated monoamine release that is not altered by clonidine.

The pharmacokinetic and other pharmacodynamic interactions between clonidine and MDMA need to be considered in the interpretation of the present findings. First, MDMA metabolism involves CYP2D6-mediated O-demethylation to 3,4-dihydroxymethamphetamine and N-demethylation to MDA by CYP2B6 and 3A4 (Segura et al., 2005). Clonidine is not known to affect CYP function. As expected, clonidine did not alter the plasma-concentration time curves for MDMA or MDA in the present study. Second, both clonidine and MDMA bind to $\alpha_2$-adrenergic receptors (Battaglia et al., 1988; Lavelle et al., 1999), and some $\alpha_2$ agonistic actions in the peripheral NE system have been documented for MDMA in vitro (Lavelle et al., 1999). However, in contrast to clonidine, MDMA increased plasma NE levels and blood pressure in the present and previous studies (Dumont et al., 2009; Hysek et al., 2011), indicating that the $\alpha_2$ agonistic effects of MDMA are not relevant for its main action in humans or are outweighed by the transporter-mediated release of NE and other monoamines.

Our study has a few limitations. First, only single doses of MDMA and clonidine were used. A dose-response study was not feasible because we did not want to expose our subjects to more than two doses of MDMA in a crossover design. The doses of both drugs were selected in the upper dose range, and both drugs produced marked effects. Unknown is whether clonidine affects the response to low doses of MDMA. Second, the clinical effects of clonidine may be attributable to actions at other binding sites. For example, clonidine binds to the imidazoline binding site with very high affinity (Buccafusco et al., 1995), and this binding site is also involved in the mediation of the pressure-lowering effects of clonidine (Ernsberger et al., 1990).

The present study further characterized the pharmacodynamic and pharmacokinetic effects of a single dose of 125 mg MDMA in healthy male and female subjects with no or only single previous MDMA use. MDMA produced cardiovascular-stimulant effects, positive
mood, emotional stimulation, and extroversion, confirming previous studies in healthy subjects (Liechti et al., 2000; Dumont and Verkes, 2006; Hysek et al., 2010; Hysek et al., 2011) and MDMA users (Farre et al., 2007; Tancer and Johanson, 2007; Kolbrich et al., 2008). The study documented the phenomenon of rapid acute tolerance to the effects of MDMA. The $E_{\text{max}}$ in blood pressure was observed within 60-120 min and $C_{\text{max}}$ was reached within 120-180 min following MDMA administration. Additionally, the plasma levels of MDMA remained close to $C_{\text{max}}$ for several hours, whereas the pharmacodynamic effects returned to baseline more rapidly. Furthermore, half-maximal effects ($EC_{50}$) of MDMA on blood pressure were observed at plasma concentrations of MDMA of approximately 50 ng/ml, which was four-fold lower than the $C_{\text{max}}$ of MDMA. Acute tolerance to the effects of psychostimulant drugs, including MDMA, cocaine, and nicotine, has previously been described (Van Dyke et al., 1978; Porchet et al., 1987; Hysek et al., 2011). This pharmacodynamic tolerance could be attributable to receptor or transporter downregulation or desensitization (Melbohm and Derendorf, 1997; Robertson et al., 2009), the more rapid distribution of drug to the brain than to venous blood (Porchet et al., 1987), or the functional depletion of presynaptic monoamine stores so that no more transmitter can be released despite high concentrations of MDMA.

In summary, our findings support the hypothesis that the effects of MDMA in humans do not depend on the vesicular release of NE but on transporter-mediated monoamine release. The clinical implications of the present study are that clonidine could be of limited use in the treatment of hypertensive reactions in psychostimulant users. In contrast, the lack of an effect of clonidine on the euphoria produced by MDMA does not indicate a role for $\alpha_2$-adrenergic receptor agonists in the prevention of psychostimulant dependence, despite their utility in the treatment of withdrawal from drugs of abuse.
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Authorship Contributions

Participated in research design: Hysek, Liechti

Conducted experiments: Hysek, Brugger, Simmler, Bruggisser, Donzelli, Grouzmann, Hoener

Performed data analysis: Hysek, Simmler, Liechti

Wrote or contributed to the writing of the manuscript: Hysek, Liechti
References


Footnotes

Conflict of interest: The authors declare no conflict of interest.

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Figure Legends

**Fig. 1.** Plasma concentration changes in (a) norepinephrine and (b) epinephrine. Values are expressed as mean ± SEM changes from baseline in 16 subjects. (a) 3,4-Methylenedioxymethamphetamine (MDMA) increased the plasma level of norepinephrine. Clonidine prevented the MDMA-induced increase in norepinephrine but also reduced the concentration of norepinephrine compared with placebo. (b) The drug effects on plasma epinephrine levels were not significant.

**Fig. 2.** Physiologic effects. Values are expressed as mean ± SEM changes from baseline in 16 subjects. Clonidine was administered at t = -1 h. 3,4-Methylenedioxymethamphetamine (MDMA) was administered at t = 0 h. (a) Clonidine reduced the MDMA-induced elevation in blood pressure to the same extent as it reduced blood pressure compared with placebo. Clonidine had no significant effects on MDMA-induced elevations in (b) heart rate and (c) body temperature.

**Fig. 3.** Time course of subjective Visual Analog Scale (VAS) ratings. Values are expressed as mean ± SEM of % maximal values in 16 subjects. 3,4-Methylenedioxymethamphetamine (MDMA) increased scores on all scales. Although clonidine produced tiredness, it only weakly and nonsignificantly affected the pronounced subjective responses to MDMA.

**Fig. 4.** Mood effects in the Adjective Mood Rating Scale (AMRS). Values are expressed as mean ± SEM of AMRS score changes from baseline in 16 subjects. 3,4-Methylenedioxymethamphetamine (MDMA) produced emotional excitation, well-being, extroversion, and dreaminess. It increased activity at the beginning of the session and inactivation toward the end of the session. Clonidine produced inactivation but did not affect responses to MDMA on any of the scales.
Fig. 5. 5-Dimensions Altered States of Consciousness (5D-ASC) scale. Values are expressed as mean ± SEM in 16 subjects. MDMA markedly increased scores on the oceanic boundlessness (OB), anxious ego dissolution (AED), visionary restructuralization (VR), and vigilance reduction (VIR) dimensions and on most subscales compared with placebo (*p < 0.05, **p < 0.01, ***p < 0.001). Clonidine did not change MDMA’s effect on any of the ASC dimensions and reduced MDMA’s effect on only one of the subscales (*p < 0.05). The main effects of the drug in the analysis of variance were significant for the sum score and all dimensions of the scale (F_{3,45} = 26.96, 25.52, 9.70, 20.49, 5.20, and 13.70 for the ASC sum score, OB, AED, VR, auditory alterations [AA], and VIR, respectively; all p < 0.001 with the exception of VIR: p < 0.01).

Fig. 6. Pharmacokinetics of (a) 3,4-methylenedioxyamphetamine (MDMA) and (b) 3,4-methylenedioxyamphetamine (MDA). The values are expressed as mean ± SEM in 16 subjects. Clonidine was administered at t = -1 h, and MDMA was administered at t = 0 h. (c) MDMA effects on blood pressure plotted against simultaneous plasma MDMA concentrations. The values are expressed as the means of the changes from baseline in 16 subjects, with SEM values omitted for clarity. The time of sampling is noted next to each point in minutes or hours after MDMA administration. Clonidine produced a downward shift in the pressure response-concentration curve of MDMA. The magnitude of the shift was similar to the pressure-lowering effect of clonidine alone compared with placebo. (d) Pharmacokinetic-pharmacodynamic (PK-PD) relationship. Values are expressed as individual MDMA concentration-effect data pairs for ascending concentrations for placebo-MDMA (black squares) and clonidine-MDMA (gray circles). Notice the large interindividual variance in the response to MDMA. The solid lines show the fit of a sigmoid E_{max} model to the pooled observed data. Dashed lines indicate 95% confidence intervals of the estimation error. Clonidine produced a downward shift of the concentration-pressure effect curve of
MDMA, indicating that it reduced the effect of MDMA at all concentrations. The magnitude of the downward shift was similar to the blood pressure-lowering effect of clonidine compared with placebo. Thus no interaction effect was found between clonidine and MDMA on blood pressure, and the effects of the two drugs were additive. Clonidine did not affect the EC$_{50}$ value of the concentration-pressure effect curve of MDMA. The EC$_{50}$ values (mean [95% confidence interval]) were 44 ng/ml (14-74 ng/ml) and 66 ng/ml (41-91 ng/ml) for placebo-MDMA and clonidine-MDMA, respectively.
## TABLE 1. Mean ± SEM values and statistics of pharmacodynamic effects.

<table>
<thead>
<tr>
<th></th>
<th>Placebo-placebo (mean ± SEM)</th>
<th>Clonidine-placebo (mean ± SEM)</th>
<th>Placebo-MDMA (mean ± SEM)</th>
<th>Clonidine-MDMA (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Circulating catecholamines</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Epinephrine (nM) E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.01 ± 0.02</td>
<td>-0.02 ± 0.03</td>
<td>0.17 ± 0.05</td>
<td>0.12 ± 0.03</td>
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<tr>
<td>Norepinephrine (nM) E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>-0.24 ± 0.10</td>
<td>-0.52 ± 0.10</td>
<td>0.41 ± 0.06</td>
<td>-0.04 ± 0.08</td>
</tr>
<tr>
<td><strong>Physiologic effect</strong></td>
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<td></td>
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<tr>
<td>SBP (mm Hg) E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>5.4 ± 3.1</td>
<td>-5.8 ± 2.2</td>
<td>36.6 ± 2.48</td>
<td>27.5 ± 3.3</td>
</tr>
<tr>
<td>AUEC&lt;sub&gt;0-6&lt;/sub&gt;</td>
<td>736.8 ± 16.0</td>
<td>654.2 ± 21.5</td>
<td>840.3 ± 17.1</td>
<td>807.5 ± 18.4</td>
</tr>
<tr>
<td>DPB (mm Hg) E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>3.0 ± 1.8</td>
<td>-4.5 ± 2.5</td>
<td>20.8 ± 2.2</td>
<td>15.3 ± 2.4</td>
</tr>
<tr>
<td>AUEC&lt;sub&gt;0-6&lt;/sub&gt;</td>
<td>438.2 ± 9.7</td>
<td>371.9 ± 12.3</td>
<td>505.8 ± 9.9</td>
<td>479.7 ± 10.8</td>
</tr>
<tr>
<td>MAP (mm Hg) E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>2.2 ± 1.7</td>
<td>-6.4 ± 1.9†</td>
<td>25.0 ± 2.2</td>
<td>18.2 ± 2.5</td>
</tr>
<tr>
<td>AUEC&lt;sub&gt;0-6&lt;/sub&gt;</td>
<td>537.7 ± 10.5</td>
<td>466.0 ± 14.7</td>
<td>616.9 ± 11.0</td>
<td>589.0 ± 12.0</td>
</tr>
<tr>
<td>Heart rate (beats/min) E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>2.8 ± 2.5</td>
<td>5.9 ± 2.9</td>
<td>26.0 ± 3.2</td>
<td>25.0 ± 5.6</td>
</tr>
<tr>
<td>AUEC&lt;sub&gt;0-6&lt;/sub&gt;</td>
<td>402.3 ± 13.9</td>
<td>389.6 ± 12.4</td>
<td>472.3 ± 15.7</td>
<td>460.0 ± 17.5</td>
</tr>
<tr>
<td>Body temperature (ºC) E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.7 ± 0.1</td>
<td>0.4 ± 0.1††</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>AUEC&lt;sub&gt;0-6&lt;/sub&gt;</td>
<td>222.3 ± 0.7</td>
<td>221.7 ± 0.6</td>
<td>223.0 ± 0.6</td>
<td>223.2 ± 0.6</td>
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<tr>
<td><strong>Visual Analog Scale (%max)</strong></td>
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<tr>
<td>Any drug effect E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.9 ± 0.9</td>
<td>16.8 ± 6.2†††</td>
<td>89.6 ± 4.0†††</td>
<td>81.56 ± 6.9††</td>
</tr>
<tr>
<td>Good drug effect E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.0 ± 0.0</td>
<td>3.2 ± 1.7††††</td>
<td>91.8 ± 4.4†††</td>
<td>79.94 ± 7.2††</td>
</tr>
<tr>
<td>Bad drug effect E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.0 ± 0.0</td>
<td>2.6 ± 2.2†</td>
<td>21.6 ± 5.8†</td>
<td>25.56 ± 6.6†</td>
</tr>
<tr>
<td>Drug liking E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.0 ± 0.0</td>
<td>2.4 ± 2.1†††</td>
<td>91.1 ± 4.6†††</td>
<td>80.94 ± 6.8††</td>
</tr>
<tr>
<td>Drug high E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.0 ± 0.0</td>
<td>0.6 ± 0.4†††</td>
<td>85.6 ± 6.7***</td>
<td>75.38 ± 8.0***</td>
</tr>
<tr>
<td>Stimulated E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.0 ± 0.0</td>
<td>0.4 ± 0.4††††</td>
<td>70.7 ± 7.9***</td>
<td>64.38 ± 9.3***</td>
</tr>
<tr>
<td>Tiredness E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>24.7 ± 6.7</td>
<td>62.8 ± 8.1††††</td>
<td>55.4 ± 7.2**</td>
<td>53.81 ± 7.4*</td>
</tr>
<tr>
<td>Closeness to others E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.4 ± 0.4</td>
<td>0.0 ± 0.0††</td>
<td>23.4 ± 4.6***</td>
<td>24.06 ± 4.7***</td>
</tr>
<tr>
<td>Open E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0†††</td>
<td>30.3 ± 4.4***</td>
<td>30.2 ± 4.9***</td>
</tr>
<tr>
<td><strong>Adjective Mood Rating Scale (score)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Emotional excitation E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>-1.0 ± 0.7</td>
<td>-0.3 ± 0.5††††</td>
<td>4.5 ± 1.1***</td>
<td>4.6 ± 0.9†</td>
</tr>
<tr>
<td>Well-being E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.8 ± 0.9</td>
<td>-0.4 ± 1.0††††</td>
<td>6.4 ± 1.5**</td>
<td>6.5 ± 1.1**</td>
</tr>
<tr>
<td>Extroversion E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>1.6 ± 0.5</td>
<td>-0.8 ± 0.4††††</td>
<td>3.3 ± 0.7</td>
<td>3.9 ± 0.8*</td>
</tr>
<tr>
<td>Dreaminess E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.6 ± 0.5</td>
<td>1.9 ± 0.6††</td>
<td>4.5 ± 0.6***</td>
<td>3.4 ± 0.7**</td>
</tr>
<tr>
<td>Activity E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.4 ± 0.6</td>
<td>-0.9 ± 1.0</td>
<td>2.1 ± 1.5</td>
<td>3.4 ± 1.0</td>
</tr>
<tr>
<td>Inactivation E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>1.9 ± 1.4</td>
<td>10.7 ± 2.0**</td>
<td>8.8 ± 2.3*</td>
<td>7.9 ± 1.8*</td>
</tr>
<tr>
<td>Anxiety-depression E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>-0.4 ± 0.4</td>
<td>0.4 ± 1.6</td>
<td>0.8 ± 0.4</td>
<td>2.1 ± 0.7†</td>
</tr>
<tr>
<td><strong>State-Trait Anxiety Inventory (state scale score)</strong></td>
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<td></td>
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<tr>
<td>Emotional excitation E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>0.1 ± 1.4</td>
<td>0.6 ± 1.2</td>
<td>3.7 ± 1.7</td>
<td>4.8 ± 2.1</td>
</tr>
</tbody>
</table>

List of complaints (total score)

- Acute adverse effects at 3 h: 0.6 ± 0.8 1.2 ± 0.6*** 10.3 ± 1.7*** 12.7 ± 1.9*** 24.48 0.001
- Subacute adverse effects at 24 h: -1.1 ± 0.6 0.1 ± 0.6 2.1 ± 1.2* 3.9 ± 1.3*** 7.08 0.001

Values are expressed as mean ± SEM changes from baseline of 16 subjects. AUEC, area under the effect-time curve; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; NS, not significant. *p < 0.05, **p < 0.01, ***p < 0.001, compared with placebo-placebo. †p < 0.05, ††p < 0.01, †††p < 0.001, compared with placebo-MDMA.
TABLE 2. Pharmacokinetic parameters of MDMA and MDA.

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0-6&lt;/sub&gt; (ng/ml·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Placebo-MDMA</td>
<td>238 ± 10</td>
<td>2.5 ± 0.2</td>
<td>7.7 ± 0.7</td>
<td>1021 ± 35</td>
</tr>
<tr>
<td>Clonidine-MDMA</td>
<td>231 ± 11</td>
<td>2.5 ± 0.1</td>
<td>9.4 ± 0.9</td>
<td>1032 ± 51</td>
</tr>
<tr>
<td>MDA</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Placebo-MDMA</td>
<td>10.9 ± 0.7</td>
<td>5.2 ± 0.3</td>
<td>NA</td>
<td>42.3 ± 3.1</td>
</tr>
<tr>
<td>Clonidine-MDMA</td>
<td>11.2 ± 0.8</td>
<td>5.1 ± 0.4</td>
<td>NA</td>
<td>44.3 ± 3.4</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 16 healthy subjects. AUC, area under the plasma concentration-time curve; C<sub>max</sub>, maximum plasma concentration; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; NA, not assessed; t<sub>1/2</sub>, terminal elimination half-life; t<sub>max</sub>, time to maximum plasma concentration.
Figure 3

- Any drug effect
- Good drug effect
- Bad drug effect
- Drug liking
- Drug high
- Stimulated
- Tiredness
- Closeness
- Open

Legend:
- Placebo-placebo
- Clonidine-placebo
- Placebo-MDMA
- Clonidine-MDMA
Figure 4

- Emotional excitation
- Well-being
- Extroversion
- Dreaminess
- Activity
- Inactivation

Legend:
- Placebo-placebo
- Clonidine-placebo
- Placebo-MDMA
- Clonidine-MDMA
Figure 5