MDMA („Ecstasy“) in Tiermodellen des Morbus Parkinson

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Inhaltsverzeichnis

1 Einleitung

Aufgrund der demographischen Entwicklung wird die Erforschung neurodegenerativer Alterserkrankungen auch in den kommenden Jahren an Bedeutung zunehmen. Neben der Alzheimer-Krankheit ist vor allem die Parkinson-Krankheit eine der häufigsten Erkrankungen des fortgeschrittenen Alters. Durch die komplexen Vorgänge im neuronalen Netzwerk Gehirn stellen neuropharmakologische und insbesondere verhaltenspharmakologische Untersuchungsmethoden, die in einem Modellorganismus durchgeführt werden eine Möglichkeit dar, die Ursachen der Krankheit einzugrenzen und mögliche Therapien zu entwickeln.

Ein weiteres wichtiges Themengebiet in der neuropharmakologischen Forschung beschäftigt sich mit den Ursachen und Mechanismen der Suchtentwicklung. Hierbei erfuhr in den letzten Jahren, neben der Erforschung „klassischer" Suchtmittel wie zum Beispiel Alkohol oder Cannabis, die Erforschung so genannter „Partydrogen", zu denen auch Ecstasy zählt, einige Bedeutung. Viele dieser Substanzen stehen im Verdacht, süchtig zu machen oder zumindest Sucht erzeugendes Verhalten zu fördern.

Ein kurioser Zufall führte zur Entdeckung, dass Ecstasy eine gute symptomatische Anti-Parkinson-Wirkung besitzt (Margolis, 2001). In der vorliegenden Arbeit soll diese Wirkung in Tiermodellen verifiziert werden und der Mechanismus der guten symptomatischen Anti-Parkinson-Wirkung von MDMA durch verhaltenspharmakologische Methoden näher charakterisiert werden.

1.1 Morbus Parkinson

Die Bezeichnung Parkinson-Krankheit oder auch Morbus Parkinson oder Paralysis agitans geht auf den Londoner Arzt James Parkinson zurück, der 1817 diese Symptome erstmals als ein zusammengehöriges Krankheitsbild erkannte. Er bezeichnete diese Erkrankung als Schüttellähmung. Der Begriff ist insofern irreführend, als eine echte Lähmung nicht zu den Symptomen der Krankheit gehört und das Schütteln (Tremor) nicht bei allen Patienten auftritt.

Die Parkinson-Krankheit ist eine der häufigsten Erkrankungen des zentralen Nervensystems. Die Erkrankung beginnt in der Mehrzahl der Fälle zwischen dem 50. und 65. Lebensjahr; es

können aber auch jüngere Patienten betroffen sein. Frauen und Männer sind etwa gleich häufig erkrankt. Bei der überwiegenden Anzahl der Patienten ist es bislang nicht möglich, eine Ursache zu finden. Man spricht dann vom idiopathischen Parkinson-Syndrom.

Neben der primären oder idiopathischen Form treten seltener auch sekundäre Formen auf wie die postencephalitische, toxische (z.B. durch Mangan, Kohlenmonoxid, Methylalkohol), medikamentöse (durch Neuroleptika) und Formen nach Schädel-Hirn-Trauma oder wiederholten Kopfschlägen bei Boxern.

Beim idiopathischen Morbus Parkinson handelt es sich um eine progressive neurodegenerative Erkrankung, die vor allem das dopaminerge mesotelencephale System betrifft. Makroskopisch findet man eine Verblassung der Hirnregion Substantia nigra bedingt durch den Verlust pigmentierter dopaminerger Neuronen. Der Zellverlust wird begleitet von einer Gliose und zytoplasmatischen Einschlusskörpern in den verbliebenen Zellen, den Lewy-Körpern. Hauptbestandteil der Lewy-Körper ist α-Synuclein (Spillantini et al., 1997), dessen Mutation bei einigen familiären Formen des Morbus Parkinson die Ursache darstellt. Symptome lassen sich erst feststellen, wenn bereits circa 80% der Neuronen degeneriert sind.

Klinische Hauptsymptome sind Akinesie (Bewegungslosigkeit) beziehungsweise Hypokinesie (Bewegungsarmut), Rigor (Steifheit der Muskulatur) und Ruhetremor (Muskelzittern). Die Akinese ist eine Verlangsamung aller Bewegungsvorgänge. Der Impuls, eine bestimmte Bewegung auszuführen, kann erst nach längerer Anlaufzeit verwirklicht werden. Auch die Geschwindigkeit des Bewegungsablaufs ist vermindert. Bei der voll ausgeprägten Parkinson-Krankheit äußert sich die Akinesie besonders in einer Störung des Gangbildes. Den Patienten fällt es schwer, einen ersten Schritt zu tun, einmal in Bewegung, fällt ihnen aber auch das Anhalten oder eine Richtungsänderung ebenfalls schwer. Während bei einem Teil der Patienten das Zittern sehr stark ausgeprägt ist, kann es bei anderen völlig fehlen. Der Ruhetremor hat eine Frequenz von 3 - 6 Schlägen pro Sekunde und hört auf, sobald mit einer Bewegung der betroffenen Extremität begonnen wird. Zusätzlich zu diesen typischen motorischen Störungen leiden Parkinson-Patienten häufig unter vegetativen Symptomen wie Seborrhoe, Speichelfluß, orthostatische Hypotonie sowie trophischen Störungen der Haut, Stimmungslabilität, Melancholie, Verlangsamung der Denkprozesse und Nachlassen der Sexualfunktion (Birkmayer & Danielczyk, 1993).

Da die exakte Ursache des idiopathischen Morbus Parkinson bislang nicht bekannt ist, existiert bis heute keine kausale Therapie der Krankheit. Schon im vorigen Jahrhundert wurden die Symptome der Krankheit mit Belladonna (Tollkirschenextrakt) behandelt. Auch Anticholinergika werden schon längere Zeit in der Therapie eingesetzt. Ein Durchbruch in der

Behandlung war die Verabreichung der Dopamin-Vorstufe L-Dihydroxyphenylalanin (L-DOPA) im Jahr 1961. 1966 ergab sich ein weiterer Durchbruch durch die zusätzliche Verwendung eines Hemmers der DOPA-Decarboxylase. Die heutige Standardtherapie besteht im Ersatz des fehlenden Neurotransmitters Dopamin und Hemmung der DOPA-Decarboxylase.

Die längerfristige Behandlung mit L-DOPA, der Vorstufe des Dopamins, führt häufig zur Entwicklung von Dyskinesien, außerdem sprechen die Patienten mit Fortschreiten der Krankheit oft nicht mehr auf die Medikation an. Auch andere Medikamente (zum Beispiel Dopaminrezptoragonisten, Monoaminoxidase-Hemmer, Catechol-O-Methyltransferase-Hemmer, Parasympatholytika, Glutamatantagonisten) und chirurgische Methoden wie die elektrische Tiefenhirnstimulation und Läsionen spezifischer Hirnregionen stellen ausschließlich symptomatische Behandlungen dar und verhindern das Fortschreiten der Krankheit nicht. Über den längerfristigen Erfolg von stereotaktischen Implantationen embryonaler Stammzellen oder Pumpen für Wachstumsfaktoren gibt es noch keine zuverlässigen Aussagen.

1.2 Tiermodelle des Morbus Parkinson

Ein Forschungsinstrument zur Klärung der pathogenen Mechanismen des Morbus Parkinson sind Tiermodelle. Eine Gruppe von Tiermodellen stellen transgene Mäuse dar, die einerseits mutiertes humanes α-Synuclein oder die Wildtyp-Form von α-Synuclein überexprimieren. Ebenfalls existiert als Tiermodell eine für humanes α-Synuclein transgene Fruchtfliege (*Drosophila melanogaster*). Diese Modelle simulieren gut die Genmutationen, die bei einem sehr kleinen Anteil der Patienten mit genetisch bedingtem Morbus Parkinson vorkommen (Betarbet et al., 2002).

Degenerative und funktionelle Vorgänge im Gehirn können sich sowohl beim Menschen, als auch beim Tier, durch Veränderungen im Verhalten äußern. Weitere tierexperimentelle Ansätze basieren daher auf der Induktion von typischen Symptomen des Morbus Parkinson und der Analyse der dadurch bedingten Verhaltensänderungen. Diese Modelle verwenden selektive Neurotoxine oder Psychopharmaka zur Induktion der Symptome des Morbus Parkinson.

1.2.1 Induktion von Symptomen im MPTP-Modell

Dieses tierexperimentelle Modell basiert auf der systemischen Verabreichung von 1-Methyl-4-phenyl-1,2,3,6-tetra-hydropyridin (MPTP), was zum Auftreten von Symptomen des Morbus Parkinson bei Menschen, Katzen und Primaten führt (Zigmond & Stricker, 1989; Tipton & Singer,1993). MPTP wird in Astrozyten durch die Monoaminoxidase B zum Pyridiniumion MPP^+ transformiert. MPP^+ wird durch seine Affinität zum Dopamin Transporter selektiv in dopaminerge Neuronen aufgenommen (Javitch et al., 1985) und blockiert die mitochondriale Atmungskette der Neuronen wodurch diese degenerieren (Lai et al., 1993). Dieses Modell kann bei Ratten nicht eingesetzt werden, da diese keine Monoaminoxidase B besitzen.

1.2.2 Induktion von Symptomen im Rotenon-Modell

Rotenon ist ein verbreitetes Pestizid und blockiert wie auch MPP^+ den Komplex I der mitochondrialen Atmungskette (Betarbet et al., 2000). Es kommt dadurch zu Radikalreaktionen und Degeneration der Zellen (Bashkatova et al., 2004). In so behandelten Ratten findet eine selektive Degeneration dopaminerger Neuronen statt, die sich in Parkinson-Symptomen äußert (Alam & Schmidt, 2002, Perier et al., 2003; Sherer et al., 2003). In diesem Modell kommt es auch zur Bildung von aus α-Synuclein bestehenden Lewy-Körperchen (Höglinger et al., 2003; Sherer et al., 2003; Alam & Schmidt, 2004). Rotenon kann sowohl peripher als auch lokal angewandt werden (Alam et al., 2004).

1.2.3 Induktion von Symptomen im 6-OHDA-Modell

Das Neurotoxin 6-Hydroxydopamin (6-OHDA) war die erste chemische Substanz deren spezifische Neurotoxizität auf katecholaminerge Nervenbahnen entdeckt wurde (Ungerstedt, 1968). Die Substanz ist sehr reaktiv und überwindet nicht die Blut-Hirn-Schranke, daher kann 6-OHDA nicht systemisch angewendet werden. Nach stereotaktischer Injektion in ein bestimmtes Hirnareal zerstört es selektiv katecholaminerge Neuronen. 6-OHDA wird durch den Membrantransporter in die Nervenzelle transportiert und setzt dort Reaktionen in Gang, bei denen freie Radikale entstehen. Dadurch gehen die betroffenen Zellen zugrunde. Um nur

dopaminerge Neuronen zu zerstören, müssen noradrenerge Neuronen durch Behandlung mit einem Noradrenalin-Membrantransporter-Hemmer geschützt werden. Je nach Injektionsort und Applikationsvolumen werden die dopaminergen Neuronen eines bestimmten Hirngebietes (zum Beispiel im Striatum oder der Substantia nigra) oder einer Projektionsbahn (zum Beispiel im medialen Vorderhirnbündel) zerstört. Bilateral lädierte Tiere kann man auf die Symptome Rigor und Akinesie im Katalepsietest untersuchen. Bei unilateral lädierten Tieren kann man die nicht lädierte Seite als negative Kontrolle verwenden und beide Körperhälften getrennt auf bestimmte motorische Fähigkeiten untersuchen (zum Beispiel beim „Pasta reaching Test“ (Ballermann et al., 2000)). Die verminderte motorische Aktivität auf der ipsilateral zur Läsion gelegenen Körperhälfte führt zu einer typischen Kreisbewegung (Rotation) von Tieren mit ausreichend starker Läsion. Dieses Modell haben Ungerstedt und Arbuthnott (1970) zur Messung der dopaminergen und anti-parkinsonoiden Wirkungen verschiedener Pharmaka eingeführt. Substanzen, die die ipsilaterale Rotation verstärken, bewirken zumeist eine vermehrte Freisetzung von Dopamin aus den intakten Nerventerminalen. Hingegen bewirken Substanzen, die zu kontralateraler Rotation führen zumeist eine Aktivierung von postsynaptischen Dopaminrezeptoren. Die Expression dieser Rezeptoren wird auf der lädierten Körperseite aufgrund der mangelnden Dopaminausschüttung durch die präsynaptischen Nervenendigungen innerhalb weniger Tage stark aufreguliert. Eine Aktivierung der Rezeptoren führt folglich zu einer stärkeren Reaktion im ipsilateralen Striatum. Wie die nachfolgenden Aktionen im Gehirn genau ablaufen ist bis heute nicht genau geklärt; man kann jedoch aufgrund der Rotation in eine Richtung auf die Wirkungsweise einer Substanz schließen.

1.2.4 Induktion von Symptomen im Neuroleptika-Modell

Dieses Modell basiert auf einem medikamentös ausgelösten Parkinson-Syndrom, wie es bei Menschen durch Behandlung mit Neuroleptika ausgelöst werden kann. Klassischerweise wird das Neuroleptikum Haloperidol dazu benutzt, bei Ratten Symptome des Morbus Parkinson hervorzurufen. Die Induktion dieser Symptome ist im Gegensatz zur Behandlung mit Neurotoxinen reversibel.

1.2.5 Quantifizierung der Symptome bei Nagern

Kognitive Funktionen werden bevorzugt in Gedächtnistests oder Labyrinthanordnungen untersucht. Zur Charakterisierung der sensormotorischen Fähigkeiten werden der Pasta Reaching Test (Ballermann et al., 2000), der Pasta Matrix Reaching Test (Ballermann et al., 2001) oder der Skilled Reaching Test (Vergara-Aragon et al., 2003) durchgeführt. Einzelne Vorgänge beim Greifen eines Futterstückchens oder einer Pastastange werden dabei bewertet. Die motorischen Fähigkeiten der Tiere werden in Tests untersucht, die auch in der vorliegenden Arbeit angewandt wurden:
Die Symptome Rigor und Akinesie werden dabei unter dem Begriff „Katalepsie“ zusammengefasst. Katalepsie stellt eine Form des Rigor dar, die herrührt von der Koaktivierung antagonistischer Muskelgruppen (Miller et al., 1990). Um das Ausmaß der Katalepsie zu quantifizieren wird das betroffene Tier zunächst in eine bestimmte Position gebracht, die es unter normalen Umständen nicht lange freiwillig beibehalten würde. Anschließend wird die Zeit gemessen bis sich das Tier willkürlich bewegt.
Beim Open Field Test werden die Tiere in eine Box gesetzt und die Bewegungen in vertikaler und horizontaler Richtung quantifiziert.
Das Rotationsverhalten wird in unilateral lädierten Tieren untersucht. Dies ist der übliche Test, um Substanzen auf ihre Anti-Parkinson-Wirkung zu testen. Nach Applikation einer Substanz werden die Anzahl und die Richtung der horizontalen Drehbewegungen registriert.

1.3 MDMA

3,4-Methylendioxy-N-Methamphetamin (MDMA) ist unter den Namen „Ecstasy“ oder „XTC“, „X“, „M“, „E“, „rolls“, „beans“, „Clarity“, „Adam“, „lover’s speed“, „hug drug“ als Modedroge bekannt. Es handelt sich bei MDMA um ein Ring-substituiertes Amphetaminderivat (Shulgin & Nichols, 1978). Die unter den vorgenannten Namen verkauften Pillen enthalten aber meist auch eine Vielzahl von anderen Amphetaminderivaten und weitere Substanzen wie zum Beispiel Ephedrin, Paracetamol oder Koffein.
MDMA wurde 1912 von der Firma Merck als Appetitzügler entwickelt und patentiert, doch kam es nie auf den Markt. In den 70er Jahren wurde es "wieder entdeckt" und in den USA bei psychiatrischen Patienten als Medikament eingesetzt. Bald gab man ihm den populären

Decknamen "Ecstasy". Seit Ende der 80er Jahre, mit dem Entstehen der Techno-Szene, hat die seit 1986 dem Betäubungsmittelgesetz unterstellte und somit illegale Substanz, eine weite Verbreitung als so genannte "Tanz- und Partydroge" gefunden. Die Amphetamin-Derivate 3,4-Methylendioxyamphetamin (MDA), 3,4-Methylendioxymethamphetamin (MDMA), 3,4-Methylendioxy-N-ethylamphetamin (MDE) und N-Methyl-1-(1,3-benzodioxol-5-yl)-2-butananamin (MBDB) wirken aufputschend und stimulierend/anregend auf Mensch und Tier (Green et al., 1995). Sie vermitteln ein Gefühl verstärkter Energie, setzen das Schlafbedürfnis herab und wirken euphorisierend. MDMA wird in die eigenständige Substanzklasse der Entaktogene eingeteilt (Nichols, 1986), in deren Wirkungsspektrum hauptsächlich ein Effekt auffällt, der von innen her („entaktogen“) einen angenehmen emotionalen Zustand der Entspannung, Angstlösung und Freude erzeugt. Die Konsumenten („User“) berichten über ein gesteigertes, offeneres Mitteilungsbedürfnis, eine größere Kommunikationsfähigkeit und ein gesteigertes Selbstbewusstsein. Dabei empfinden sie eine besondere Harmonie mit dem Gesprächspartner. Oftmals werden optische und akustische Halluzinationen erlebt.

Eine starke psychomotorische Aktivierung wurde bei Tieren nach Gabe von MDMA beobachtet, was sich zum Beispiel in erhöhter Lokomotion und vermehrter Schnüffelaktivität äußert (Spanos & Yamamoto, 1989). Bis heute ist nicht vollständig geklärt, ob MDMA und seine Derivate zu den Suchtstoffen zu zählen sind.

Auf neurochemischer Ebene führt MDMA zur Freisetzung von Serotonin und - in geringerem Maße - von Dopamin und Noradrenalin (Schmidt et al., 1987; Yamamoto & Spanos, 1988). MDMA wird entweder durch Diffusion oder im Cotransport mit Natriumionen über den Membrantransporter in die Axonterminalen aufgenommen und hemmt die Wiederaufnahme von Serotonin in die präsynaptische Nervenendigung (Nichols et al., 1982; Steele et al., 1987; Johnson et al., 1991; Rudnick & Wall, 1992; Gudelsky & Nash, 1996). Der Transport von Serotonin wird sogar umgedreht, das heißt es kommt zur Freisetzung von Serotonin über den Membrantransporter (Hekmatpanah & Peroutka, 1990). Zusätzlich zur Beeinflussung des membranständigen Transporters behindert MDMA die Aufnahme von Monoaminen durch den vesikulären Monoamintransporter (Bogen et al., 2003; Hansen et al., 2002; Mlinar & Corradetti, 2003). Dies führt zu einem Anstieg der Serotoninkonzentration im Zytosol und zur Umkehr des membranständigen Transporters, der nun Serotonin in den Extrazellulärraum abgibt. Darüber hinaus ist bekannt, dass MDMA das entscheidende Enzym der Serotonin-Biosynthese, die Tryptophan Hydroxylase, hemmt (Schmidt & Taylor, 1987; Stone et al., 1987).

Die Freisetzung von Dopamin wird durch direkte Bindung am Transporter (Nash & Brodkin, 1991; Schmidt et al., 1987, Yamamoto & Spanos, 1988; White et al., 1996; Iravani et al., 2000) und durch indirekte Wirkungen des serotonergen auf das dopaminerge System verursacht (Brodkin et al., 1993; Schmidt et al., 1994; Gudelsky & Nash, 1996; Obradovic et al., 1996; Koch & Galloway, 1997; Shankaran & Gudelsky, 1998; Bankson & Cunningham, 2001). Hierbei wurde dem 5-HT2A-Rezeptor eine potenzierende Rolle bei der durch MDMA verursachten Dopaminfreisetzung zugesprochen (Gudelsky et al., 1994; Nash, 1990; Schmidt et al., 1994; Yamamoto et al., 1995). Im Allgemeinen wird sonst von einer positiven Wirkung einer 5-HT2-Rezeptorblockade auf die Dopaminfreisetzung berichtet (Devaud et al., 1992; Pehek et al., 1993; Schmidt et al., 1995). Neue Berichte beschreiben eine Modulation der Dopaminfreisetzung durch MDMA über den Signalweg der Proteinkinase C (Nair & Gudelsky, 2004). Die Aktivierung der Serotoninrezeptoren bewirkt eine Translokation der Proteinkinase C vom Zytosol zur Zellmembran (Kramer et al., 1995, 1997,1998). Eine Stimulation der Proteinkinase C erhöht, eine Inhibition vermindert die Dopaminfreisetzung durch MDMA (Nair &Gudelsky, 2004).

Alle Amphetaminderivate besitzen ein chirales Kohlenstoffatom. Dies ist die Ursache für teilweise völlig verschiedene pharmakologische Wirkungen. Während die S-Enantiomere der Methylendioxy-Reihe entaktogen wirken, sind die R-Enantiomere halluzinogen. Die Enantiomere besitzen unterschiedliche Bindungseigenschaften an den neurobiologisch relevanten membranständigen Proteinen der Nervenzellen wie Rezeptoren und Transportermoleküle. Des Weiteren wird ein unterschiedliches Anfluten im Gehirn und ein enantioselektiver Metabolismus vermutet. Hinsichtlich der Freisetzung von Serotonin unterscheiden sich die Enantiomere nicht, jedoch wird durch S(+)-MDMA mehr Dopamin freigesetzt (Johnson et al., 1986).

Wiederholte Gabe von MDMA ruft sowohl bei Nagern, als auch bei Affen eine Degeneration der serotonergen Axonterminalen und folglich einen Mangel an Serotonin in den Zielstrukturen serotonerger Nervenfasern hervor. Hierbei sind vor allem die feinen Neuronen betroffen, die von der dorsalen Raphe in kortikale Areale und die Basalganglien projizieren. Aufgrund der neurotoxischen Effekte ging man bisher davon aus, dass MDMA in hoher Dosierung die Entwicklung von Parkinson-Symptomen fördern könnte. Hierzu sind Fallberichte erschienen (Kuniyoshi & Jankovic, 2003). Befürchtungen, dass Ecstasy-Konsumenten ein erhöhtes Risiko haben im späteren Leben an einem Morbus Parkinson zu erkranken, wurden anfänglich durch eine Studie an Primaten geschürt (Ricaurte et al., 2002). Die Ergebnisse dieser Studie beruhten jedoch auf einem Irrtum. Eine dopaminerge

Neurotoxizität durch MDMA bei Primaten ist nicht nachgewiesen (Ricaurte et al., 2003). In Bezug auf Menschen finden sich vermehrt Berichte über Spätfolgen durch chronischen Konsum von MDMA: einerseits ein verminderter Serotoningehalt in der Zerebrospinalflüssigkeit, andererseits Berichte über psychische Veränderungen und Gedächtnisstörungen (Green et al., 2003; Cole & Sumnall, 2003; de la Torre et al., 2004). Die Mechanismen dieser Neurodegeneration sind noch nicht geklärt und zurzeit Gegenstand aktueller Forschung.

Aufgrund der bislang ungeklärten suchterzeugenden Wirkung und eines nachgewiesenen neurotoxischen Effekts von MDMA auf serotonerge Nervenzellen, kann MDMA selbst nicht für die Therapie des Morbus Parkinson eingesetzt werden. Würde man jedoch den Wirkungsmechanismus kennen, ließen sich eventuell bessere Anti-Parkinson-Medikamante entwickeln.

1.4 Fragestellung

Bisher existiert nur eine einzige episodische Darstellung einer beobachteten guten symptomatischen Anti-Parkinson-Wirkung bei einem an einer juvenilen Form des Morbus Parkinson erkrankten Menschen (Margolis, 2001). Auch wurde MDMA bisher nur in einem Tierversuch in der durch Neuroleptika induzierten Katalepsie eingesetzt (Schmidt et al., 2002). Die symptomatische Anti-Parkinson-Wirkung von MDMA ist daher noch nicht ausreichend charakterisiert. Diese Wirkung soll daher in der vorliegenden Arbeit in der Neuroleptika-induzierten Katalepsie untersucht und im Rotationsverhalten nach 6-OHDA-Läsion verifiziert werden. Auf welche neurochemischen Effekte die psychomotorische Aktivierung und die positive Wirkung sowohl bei Parkinson-Patienten, als auch im Katalepsie-Modell bei der Ratte zurückzuführen ist, ist zum gegenwärtigen Zeitpunkt noch unbekannt. Die naheliegende Erklärung, dass MDMA wie L-DOPA die Dopaminkonzentration erhöht, erscheint aus Sicht der gegenwärtig vorhandenen Literatur fragwürdig. Daher soll hier außerdem der Frage nach dem Mechanismus beziehungsweise den Mechanismen dieser positiven symptomatischen Anti-Parkinson-Wirkung von MDMA nachgegangen werden. Die Aussicht, über die Klärung der Wirkungsweise von MDMA, Rückschlüsse für die Entwicklung wirksamer Anti-Parkinsonmittel ziehen zu können, stellt eine starke Motivation dar.

1.4.1 Publikation I

LEBSANFT HB, MAYERHOFER A, KOVAR K-A, SCHMIDT WJ
Is the Ecstasy-induced ipsilateral rotation in 6-hydroxydopamine unilaterally lesioned rats dopamine independent? *J Neural Transm* (2003) 110(7):707-718.

Die Anti-Parkinson-Wirkung von MDMA wurde hier zum ersten Mal an Tieren mit einer Neurodegeneration im 6-OHDA-Modell überprüft, welches das wahrscheinlich am weitesten verbreitete Parkinson-Modell ist. Falls MDMA Rotationen in unilateral 6-OHDA-lädierten Tieren auslösen würde, wäre die nächste wichtige Frage, in welche Richtung diese erfolgen würden. Aus den Ergebnissen dieser Versuche sollten sich erste Diskussionspunkte für den möglichen Angriffspunkt von MDMA im nigrostriatalen Dopaminsystem ergeben. Verschiedene Dosierungen von MDMA wurden hierbei eingesetzt, um eine eventuelle Dosis-

Wirkungs-Beziehung aufzudecken. Die MDMA-Derivate MBDB, MDE und MDA wurden im selben Modell eingesetzt, um die unterschiedliche Effektivität dieser Substanzen vergleichen zu können und aus deren pharmakologischem Profil eventuelle Rückschlüsse auf das für die Anti-Parkinson-Wirkung essentielle Element des pharmakologischen Profils ziehen zu können. Zur Klärung des Einflusses von MDMA auf das serotonerge System wurden der selektive Serotonin-Wiederaufnahme-Hemmer Citalopram und anschließend der selektive Serotonin Synthese Inhibitor PCPA mit MDMA kombiniert eingesetzt.

1.4.2 Publikation II

LEBSANFT HB, KOHLES T, KOVAR K-A, SCHMIDT WJ.
3,4-Methylenedioxymethamphetamine counteracts akinesia enantioselectively in rat rotational behaviour and catalepsy. Angenommen bei *Synapse* (2004).

MDMA ist ein chirales Molekül. Es existieren zwei Konfigurations-Isomere, die sich wie Bild und Spiegelbild verhalten: die zwei Enantiomere R(-)-MDMA und S(+)-MDMA. Enantiomere unterscheiden sich nicht in ihrem chemischen Verhalten und - mit Ausnahme ihrer optischen Aktivität - auch nicht in physikalischen Eigenschaften. Unterschiede gibt es jedoch in ihrem biochemischen Verhalten, denn im Organismus kommt es zu Wechselwirkungen mit anderen chiralen Molekülen, bei denen die Konfiguration einen wesentlichen Einfluss hat. Die Wirkung der Enantiomere des MDMA wurde daher im Modell der Haloperidol-induzierten Katalepsie und im Rotationsverhalten nach unilateraler Läsion des medialen Vorderhirnbündels untersucht und direkt verglichen.

1.4.3 Publikation III

LEBSANFT HB, KOVAR K-A, SCHMIDT WJ.
3,4-Methylenedioxymethamphetamine and naloxone in rat rotational behaviour and open field. Eingereicht bei *European Journal of Pharmacolog,* (2004).

Das endogene Opiat Enkephalin trägt Literaturberichten zufolge (Compan et al., 2003) zum lokomotionssteigernden Effekt von MDMA in Mäusen bei. Ebenso ist von Morphin als μ-Rezeptor-Agonist bekannt, dass es explosionsartige Lokomotionssteigerungen hervorruft,

jedoch in höheren Dosierungen selbst Katalepsie hervorruft. Vor diesem Hintergrund tut sich die Frage auf nach einer eventuellen Beteiligung endogener Opiate an der Anti-Parkinson-Wirkung von MDMA. In dieser Arbeit wurde untersucht, in welchem Ausmaß eine Aktivierung von μ-Rezeptoren an den lokomotionssteigernden Effekten von MDMA bei Ratten und an der MDMA-vermittelten ipsilateralen Rotation in 6-OHDA lädierten Ratten beteiligt ist. Hierzu wurde das Verhalten naiver Ratten nach systemischer Behandlung mit MDMA, Naloxon und mit einer Kombination aus MDMA und Naloxon in einem Open Field analysiert. Des Weiteren wurden unilateral mit 6-OHDA lädierte Ratten mit MDMA und Naloxon in verschiedenen Konzentrationen behandelt und das Rotationsverhalten untersucht.

2 Zusammenfassung der in den Publikationen enthaltenen Ergebnisse

2.1 Publikation I

LEBSANFT HB, MAYERHOFER A, KOVAR K-A, SCHMIDT WJ

Is the Ecstasy-induced ipsilateral rotation in 6-hydroxydopamine unilaterally lesioned rats dopamine independent? *Journal of Neural Transmission* (2003) 110(7):707-718.

Über Fernsehen und Printmedien war vor kurzer Zeit ein Fall bekannt geworden, bei dem ein an juvenilem Morbus Parkinson erkrankter Patient durch die Selbstmedikation mit Ecstasy erstaunliche Erfolge bei der Besserung seiner Symptome erzielen konnte. Erste Versuche mit Ratten bei der Blockade der Neuroleptika-induzierten Katalepsie durch MDMA und verschiedene MDMA-Derivate bestätigten diesen Effekt und ließen zugleich die Frage nach dem Mechanismus dieser symptomatischen Anti-Parkinson-Wirkung aufkommen. In der vorliegenden Publikation wurden daher Ratten mit 6-OHDA unilateral auf Höhe des medialen Vorderhirnbündels lädiert und das Rotationsverhalten der Tiere nach Behandlung mit MDMA, MBDB, MDE und MDA untersucht. Alle Substanzen induzierten ipsilaterale Rotationen, wobei MDA am stärksten wirkte. Die Anzahl der Drehungen, welche die Substanzen auslösten, korrelierte mit der aus der Literatur bekannten Stärke der Dopaminfreisetzung. Die Rotationen nach Gabe von MDMA waren dosisabhängig und konnten durch gleichzeitige Behandlung mit dem Serotonin-Wiederaufnahme-Hemmer Citalopram abgeschwächt werden. Citalopram alleine konnte keine Rotationen auslösen. Auch eine Vorbehandlung der Tiere mit dem selektiven Serotonin-Synthese-Hemmer PCPA konnte die durch MDMA ausgelösten ipsilateralen Rotationen nur leicht abschwächen. Daraus ergibt sich die Schlussfolgerung, dass die Wirkungen von MDMA nicht vollständig durch die Freisetzung von Serotonin oder durch die Wirkung auf das dopaminerge System zurückzuführen sind.

2.2 Publikation II

LEBSANFT HB, KOHLES T, KOVAR K-A, SCHMIDT WJ.
3,4-Methylenedioxymethamphetamine counteracts akinesia enantioselectively in rat rotational behaviour and catalepsy. Angenommen bei *Synapse* (2004).

In den dieser Publikation zugrunde liegenden Experimenten wurde die symptomatische Anti-Parkinson-Wirkung der beiden Enantiomere und des Racemats von MDMA im Rotationsverhalten und in der Haloperidol-induzierten Katalepsie verglichen. Die Katalepsietests wurden mit zuvor naiven, unlädierten Tieren durchgeführt. Parkinson-Symptome wurden durch intraperitoneale Verabreichung von 0,5 mg pro kg Körpergewicht Haloperidol induziert und wiederholt als Abstiegslatenz von einer horizontal angeordneten Stange und von einem vertikal angeordneten Gitter. Sowohl R(-)-MDMA, als auch S(+)-MDMA waren gegen die Haloperidol-induzierte Katalepsie wirksam. Der Effekt bei der racemischen Form des MDMA war jedoch deutlicher ausgeprägt als bei den Enantiomeren. Zur Untersuchung des Rotationsverhaltens wurden männliche Sprague-Dawley-Ratten unilateral mit 6-OHDA auf Höhe des medialen Vorderhirnbündels lädiert. S-MDMA in einer Dosierung von 5 mg pro kg Körpergewicht induzierte ipsilaterale Rotationen, R-MDMA war weit weniger wirksam. S-MDMA, zusätzlich zu R-MDMA gegeben, steigerte die Anzahl der ipsilateralen Rotationen deutlich.
Das S(+)-Enantiomer des MDMA stellt demzufolge die effektivere Substanz in Bezug auf die symptomatische Anti-Parkinson-Wirkung dar. Jedoch können sich die Effekte beider Enantiomere gegenseitig verstärken.

2.3 Publikation III

LEBSANFT HB, KOVAR K-A, SCHMIDT WJ.

3,4-Methylenedioxymethamphetamine and naloxone in rat rotational behaviour and open field. Eingereicht bei *European Journal of Pharmacology* (2004).

Literaturberichten zufolge spielen endogene Opiate eine Rolle bei durch MDMA ausgelöstem lokomotorischem Verhalten bei Mäusen (Compan et al, 2003). Ob dies auch bei Ratten der Fall ist, wurde zum Einen im Lokomotionsverhalten normaler Ratten und zum Anderen im Rotationsverhalten unilateral 6-OHDA-lädierter Ratten untersucht. Im Gegensatz zu den erwähnten Arbeiten mit Mäusen blockierte Naloxon (10 mg/kg, s.c.) bei unlädierten Ratten nicht die durch MDMA (5 mg/kg, s.c.) induzierte Hyperaktivität im Open Field. Männliche Sprague-Dawley-Ratten wurden unilateral mit 6-OHDA auf Höhe des medialen Vorderhirnbündels lädiert. R/S-MDMA (5 mg/kg, s.c.) induzierte ipsilaterale Rotationen. Naloxon (2,5,10 mg/kg, s.c.) induzierte im Gegensatz zu MDMA keine ipsilateralen Rotationen, reduzierte aber die Anzahl der durch MDMA induzierten Rotationen in unilateral lädierten Tieren. Dieser Effekt war nicht dosisabhängig. Endogene Opiate spielen daher eine Rolle bei der Wirkung von MDMA im Rotationsverhalten der Ratte, nicht jedoch bei der durch MDMA induzierten Hyperaktivität.

3 Diskussion

In der vorliegenden Arbeit wurden verschiedene Experimente durchgeführt, alle mit dem gemeinsamen Ziel, die gute symptomatische Anti-Parkinson-Wirkung von MDMA in Tiermodellen zu überprüfen und feststellbare Effekte näher zu charakterisieren. Dies ist eine Voraussetzung zur Klärung des Mechanismus für diesen Effekt und für die Entwicklung von neuen und besseren Anti-Parkinsonmitteln.
Die symptomatische Anti-Parkinson-Wirkung von MDMA in den Tiermodellen Rotation nach unilateraler 6-OHDA-Läsion und Neuroleptika-induzierte Katalepsie sowie die lokomotionssteigernde Wirkung im Open Field wurde in der vorliegenden Arbeit demonstriert.
Nach wiederholter Gabe und erfolgtem Test auf Rotationsverhalten wurde unter Saline-Behandlung ebenfalls eine leichte ipsilaterale Rotation festgestellt (I). Dies ist bereits für das Rotationsverhalten nach mehrfacher Behandlung mit Rotationen auslösenden Substanzen als konditionierter Effekt beschrieben worden (Carey 1986a,b;1988). Bei MPTP-behandelten Krallenaffen bewirkte MDMA eine anfängliche und vorübergehende Besserung der motorischen Behinderungen aber nachfolgend eine Verschlechterung und eine langandauernde Reduktion der motorischen Aktivität (Iravani et al., 2003). Diese negativen Effekte konnten in der vorliegenden Arbeit bei Ratten nicht beobachtet werden.

Der Test auf Rotationsverhalten ist einer der wichtigsten Tests bei der Suche nach neuen potentiellen Anti-Parkinson-Mitteln. Um das Ausmaß der Läsion durch 6-OH-Dopamin zu überprüfen, wurden die Ratten zunächst mit Apomorphin behandelt. Dies führte erwartungsgemäß zu Rotationen in kontralateraler Richtung. Man kann die Tiere daher als hemi-parkinsonoid bezeichnen (Hefti et al., 1980; Hudson et al., 1993; Barneoud et al., 1995; Przedborski et al., 1995). Um Rotationen kontralateral zur Seite der Läsion zu induzieren, müssen die entsprechenden Substanzen an den postsynaptischen Rezeptoren wirksam sein. Für die Dopaminrezeptoren besitzt MDMA auch eine sehr geringe Affinität (Battaglia et al., 1988). Hingegen sind ipsilaterale Rotationen ein Hinweis auf eine präsynaptische Dopaminfreisetzung.
MDMA war im Modell der unilateralen 6-OHDA-Läsion wirksam und verursachte nach systemischer Verabreichung ipsilaterale Rotationen der Tiere. Die Rotationen nach subkutaner Verabreichung von MDMA waren dosisabhängig und reproduzierbar. Ein

präsynaptischer Wirkmechanismus in Bezug auf das dopaminerge System scheint daher für MDMA und seine Derivate wahrscheinlich (I, II, III). Wie die Dopaminfreisetzung zustande kommt, lässt sich leider mit dem Rotationsmodell zunächst nicht ergründen. Mehrere andere Neurotransmittersysteme beeinflussen nachgewiesenermaßen das dopaminerge System und können so auch indirekt zu einer Dopaminfreisetzung aus nigrostriatalen Neuronen führen. Man kann also von einem vornehmlich präsynaptischen Wirkungsmechanismus von MDMA und seinen Derivaten ausgehen. Ipsilaterale Rotationen sind vor allem für Amphetamin charakteristisch, das auch einige Gemeinsamkeiten im Verhalten mit MDMA aufweist. Allerdings unterscheiden sich die Substanzen auch in anderen Verhaltens- und neurochemischen Effekten. Wie Amphetamin führt auch MDMA zu einer Freisetzung von Dopamin (Yamamoto & Spanos, 1988; Nash, 1990; Nash & Nichols, 1991; Colado & Green, 1994; White et al., 1994, 1996; Gudelsky & Nash, 1996; Kankaanpaa et al., 1998; Bankson & Cunningham, 2001), jedoch ist das Verhältnis von Serotoninfreisetzung zu Dopaminfreisetzung bei MDMA viel größer als bei Amphetamin (Crespi et al., 1997).

In Bezug auf die psychisch aktivierenden Eigenschaften wird dem mesolimbischen Dopaminsystem eine Rolle zugesprochen, da eine Dopamin-Entleerung durch 6-OHDA die Verhaltenseffekte von MDMA abschwächte (Gold et al., 1989). Es gibt auch Hinweise, dass MDMA den membranständigen Dopamin-Wiederaufnahme-Transporter inhibiert (Mezger et al., 1998). MDMA hemmt nachgewiesenermaßen die Aufnahme von Monoaminen in Vesikel (Hansen et al., 2002; Bogen et al., 2003; Mlinar & Corradetti, 2003). Dies könnte zu einer erhöhten Konzentration von Serotonin und/oder Dopamin führen, das dann über den in seiner Richtung umgedrehten membranständigen Transporter freigesetzt wird. Ein solcher Mechanismus ist bereits für die durch Amphetamin induzierte Dopaminfreisetzung im Gespräch (Fon et al., 1997; Jaber et al., 1997; Wang et al., 1997; Jones et al., 1998).

Eine weitere Hypothese geht von einem Diffusions-Austauschmechanismus für Serotonin und Dopamin aus, dessen Ursache die Hemmung der Monoaminoxidase durch MDMA ist. Im Gegensatz zu Amphetamin ist die Hemmung dieses Enzyms aber für MDMA nicht stereoselektiv (Mantle et al., 1976; Kokotos Leonardi & Azmitia, 1994). Die Aktivität von MDMA im Rotationsverhalten und der Katalepsie ist jedoch stereoselektiv (II), eine Hemmung der Monoaminoxidase scheidet daher als essentieller Faktor für die symptomatische Anti-Parkinson-Wirkung von MDMA aus.

Die Stärke des ausgelösten Rotationsverhaltens der Derivate von MDMA stieg in der folgenden Reihenfolge an: MBDB, MDE, MDMA, MDA (I). Dies entspricht auch der

ansteigenden Reihenfolge der durch diese Substanzen verursachten Dopaminfreisetzung (Nash &Nichols, 1991; Johnson et al., 1986). Ein erhöhter extrazellulärer Dopaminspiegel ist auch für S-MDMA beschrieben, das im Rotationsverhalten und der Katalepsie stärker wirksam war (II). Hingegen ist R-MDMA sowohl bei der Dopaminfreisetzung, als auch im Rotationsverhalten und der Katalepsie wesentlich weniger wirksam (Yamamoto & Spanos, 1988; Hiramatsu & Cho, 1990; Nash, 1990; Gough et al., 1991; Schmidt el al, 1991; Nash &Yamamoto, 1992; Koch & Galloway, 1997).

Der beobachtete Zeitverlauf der Rotationen (I, II, III) korreliert auch gut mit einigen Literaturberichten über die durch MDMA verursachte in vivo Dopaminfreisetzung im Striatum (Nash, 1990; Nash & Yamamoto, 1992; Gough et al, 1991; Gudelsky & Nash, 1996). Für Amphetamin wurde die direkte Korrelation zwischen Dopaminfreisetzung und ipsilateralen Rotationen bereits dargestellt (Zetterstrom et al., 1986). In anderen Literaturquellen finden sich aber auch abweichende Zeitverläufe zur Dopaminfreisetzung von MDMA (Sabol & Seiden, 1998; Gudelsky et al., 1994) und eine im Zeitverlauf ähnliche Serotoninfreisetzungskurve (Shankaran & Gudelsky, 1999). Daher kann aufgrund einer zeitlichen Korrelation des Verhaltens mit der Dopaminfreisetzung nicht auf einen direkten dopaminergen Mechanismus geschlossen werden.

Der Zeitverlauf der durch MDA und MDMA induzierten Rotationen war sehr ähnlich, obwohl MDA stärker wirksam war (I). Dies ist ein Hinweis darauf, dass beide Substanzen durch die selben Mechanismen zur Dopaminfreisetzung führen könnten. MDA ist ein Metabolit von MDMA und besonders bei dem sehr gut syptomatisch Anti-Parkinson wirksamen S-MDMA (II) finden sich hohe Konzentrationen dieses Metaboliten im Kortex und Striatum (Hiramatsu & Cho, 1990; Meyer et al., 2002).

Eine zeitliche Korrelation der Dopaminfreisetzung mit der Bildung von MDA aus MDMA ist jedoch nicht vorhanden (Hiramatsu & Cho, 1990; Hiramatsu et al., 1991; Schmidt et al., 1991). Die gute symptomatische Anti-Parkinson-Wirkung von MDMA ist demzufolge der Muttersubstanz und nicht dem mehr Dopamin freisetzenden Metaboliten MDA zuzuschreiben.

Die durch den chiralen Charakter des Moleküls bedingten unterschiedlichen pharmakologischen Eigenschaften wurden sowohl im Rotationsverhalten, als auch bei der durch Haloperidol induzierten Katalepsie deutlich (II). In der vorliegenden Arbeit war das S-Enantiomer von MDMA insgesamt wirksamer in der symptomatischen Anti-Parkinson-Wirkung. Das R-Enantiomer konnte jedoch die Effekte von S-MDMA noch verstärken (II).

Am besten reproduzierbar waren in diesem Zusammenhang die Effekte von racemischem MDMA. Ein Grund für die größere symptomatisch Anti-Parkinson-Wirkung von S(+)-MDMA könnte in seiner höheren Affinität zum Serotonintransporter (Rudnick & Wall, 1992) begründet liegen. Der Serotonintransporter ist sehr wichtig für die Wirkung von MDMA in serotonergen Neuronen. Daher setzt S-MDMA auch mehr Serotonin frei (Anderson et al., 1978; Nichols et al., 1982; Cho et al, 1990; Hiramatsu & Cho, 1990; Koch & Galloway, 1997). Serotonin-Transporter-defiziente Mäuse sind unempfindlich für die lokomotionssteigernden Eigenschaften von S(+)-MDMA (Bengel et al., 1998), dies zeigt die große Bedeutung des Serotonin-Transporters für den Wirkmechanismus von MDMA und im speziellen für dessen S-Enantiomer.

Die Ursache für die größere Wirksamkeit von S(+)-MDMA könnte aber auch die erhöhte Dopaminfreisetzung nach systemischer Verabreichung sein, da es etwa fünf mal mehr Dopamin freisetzt als R(-)-MDMA (Kelland et al.,1990). Dieses Argument wird durch die Befunde gestärkt, dass die MDMA-Derivate, die am meisten Dopamin freisetzen (Nash & Nichols, 1991), auch die größte Wirksamkeit auf das Rotationsverhalten haben (I). Bei der Katalepsie verhält es sich bis auf MDA ebenso (Schmidt et al., 2002). Beim Vergleich der Enantiomere ist die in vitro Freisetzung und Wiederaufnahme-Hemmung nur für das S-MDMA beschrieben oder zumindest für dieses Enantiomer effektiver, in Bezug auf das serotonerge und noradrenerge System ergaben sich jedoch keine Unterschiede zwischen beiden Enatiomeren (Nichols et al., 1982; Johnson et al., 1986; Schmidt et al., 1987; Steele et al., 1987; Kalix et al., 1988; Mc Kenna et al., 1991).S-MDMA war auch die effektivere Substanz in Bezug auf Verhaltensweisen, die mit dopaminergen Mechanismen in Verbindung stehen (Hiramatsu et al.,, 1989; Rosecrans & Glennon, 1987; Glennon et al., 1988). In Diskriminations-Experimenten war S-MDMA ebenfalls die effektivere Substanz (Schechter, 1987; Battaglia et al., 1988; Glennon et al., 1988) und vermittelte eher eine dopaminerge Wirkung, während R-MDMA eher eine sertonerge Wirkung vermittelte (Glennon et al., 1988; Baker & Taylor, 1997; Fantegrossi et al., 2002). Vergleichbar mit der vorliegenden Arbeit war jedoch das Racemat die effektivste Substanz.

Die ipsilaterale Richtung der Rotationen spricht zunächst ebenfalls für einen dopaminergen Wirkmechanismus (I, II, III). Jedoch bestehen neben einigen Parallelen bei MDMA neurochemische Unterschiede gegenüber Amphetamin, das vor allem über dopaminerge Mechanismen wirkt. Eine Freisetzung von Dopamin durch MDMA ist unbestritten (Mc Kenna et al., 1991, White et al., 1996); auch korreliert diese Freisetzung zeitlich gut mit den Verhaltensänderungen der Tiere in den vorliegenden Experimenten.

Der selektive Serotonin-Wiederaufnahme-Hemmer Citalopram löste selbst keine Rotationen aus, schwächte aber die durch MDMA induzierten Rotationen ab (I). Daher scheint es sehr unwahrscheinlich, dass die ipsilateralen Rotationen, die MDMA induzierte, ausschließlich durch eine direkte präsynaptische Freisetzung von Dopamin verursacht wurden. Citalopram hätte dann keine Wirkung auf die durch MDMA ausgelösten Rotationen haben dürfen. Es wurde bereits gezeigt, dass Serotonin-Wiederaufnahme-Hemmer die Serotininfreisetzung durch MDMA verhindern können, die Dopaminfreisetzung bleibt jedoch unverändert (Mechan et al., 2002). Man kann daher annehmen, dass Citalopram das Wirken von MDMA in serotonergen Zellen behindert, die dopaminergen Neuronen aber nicht beeinflusst. Citalopram greift am Serotonin-Wiederaufnahme-Transporter an und verhindert das Eindringen von MDMA über diesen Transporter und die Umkehr desselben durch MDMA. Aus der Abschwächung der durch MDMA induzierten Rotationen kann man schließen, dass der Effekt von MDMA auf das Rotationsverhalten zumindest teilweise durch die Aufnahme in serotonerge Neuronen vermittelt wird. Es ist jedoch unwahrscheinlich, dass die Rotationen einfach durch Blockade der Wiederaufnahme und dadurch erhöhte extrazelluläre Serotoninspiegel ausgelöst werden, denn Citalopram selbst konnte in der vorliegenden Arbeit keine Rotationen auslösen. Im Gegensatz zu MDMA beeinflusst Citalopram das dopaminerge und noradrenerge System nicht, sondern erhöht nur den extrazellulären Serotoninspiegel (Millan et al., 2000). Andere Studien mit Citalopram zeigten, dass physiologische Effekte von MDMA beim Menschen ebenfalls teilweise durch eine Interaktion von MDMA mit dem membranständigen Serotonin-Transporter und nachfolgende Serotoninfreisetzung zustande kommen und einige Verhaltenseffekte von MDMA durch Citalopram verhindert werden (Liechti & Vollenweider, 2000; Liechti et al., 2000).

Es wurde bereits gezeigt, dass MDMA mehr Serotonin und Noradrenalin freisetzt als Dopamin (Rothmann et al., 2001). Jedoch wird ein erhöhter Serotoninspiegel im Allgemeinen nicht mit einer Anti-Parkinson-Wirkung in Verbindung gebracht. Im Gegenteil verstärken Serotoninfreisetzung oder –Wiederaufnahmehemmung eher Parkinson-Symptome im Katalepsiemodell bei der Ratte und es gibt ebenfalls Berichte über die Induktion von Parkinson-Symptomen bei Menschen (Stadtland et al., 2000). Man nimmt an, dass dies durch eine Hemmung dopaminerger Neuronen aufgrund serotonerger Mechanismen zustande kommt (Sinton et al., 1988; Kelland et al., 1990; Neal-Beliveau et al., 1993).
Serotonerge Projektionen aus den dorsalen Raphekernen innervieren alle Strukturen der Basalganglien (Lavoie & Parent, 1990). Die serotonerge Transmission bei Parkinson-

Patienten ist gestört (Hornykiewicz, 1998). Daher spielt Serotonin eine Rolle bei der Bewegungsinduktion durch die Basalganglien. Das serotonerge System ist jedoch sehr komplex organisiert. Bis zum gegenwärtigen Zeitpunkt wurden 14 Serotoninrezeptoren in 7 verschiedenen Familien klassifiziert, die sich in ihrem molekularen Aufbau, ihrer Signaltransduktion und Pharmakologie unterscheiden (Barnes & Sharp, 1999). Viele dieser Rezeptoren kommen in den Basalganglien vor und besonderes Interesse kommt den Rezeptoren 5-HT1A; 5-HT1B und 5-HT2C zu, die auch für den Wirkmechanismus von MDMA eine Rolle spielen. 5-HT1A-Rezeptor- Stimulation vermittelt eine Anti-Parkinson-Wirkung, da diese als Autorezeptoren die Feuerrate der serotonergen Neuronen vermindern (Gerber et al., 1988; Neal-Beliveau et al., 1993). Für 5-HT2-Rezeptor-Agonisten wurde bereits eine Verminderung der durch Raclopride induzierten Katalepsie nachgewiesen (Wadenberg et al., 1996). Eine Beteiligung der 5-HT2-Rezeptoren wurde ferner nachgewiesen bei serotonerger Neurotoxizität, akuter Hyperthermie, Kortikosteronsekretion, Lokomotionssteigerung, Belohnung und weiteren Effekten von MDMA (Nash et al., 1988; Schmidt et al, 1990; Kehne et al., 1996; Padich et al., 1996; Fantegrossi et al., 2002). Jedoch wurde auch eine Beteiligung der 5-HT2-Rezeptoren bei der Dopaminfreisetzung diskutiert (Gudelsky et al., 1994; Schmidt et al., 1994; Yamamoto et al., 1995). Das R-Enantiomer besitzt eine höhere Affinität zu Serotoninrezeptoren als das S-Enantiomer (Lyon et al., 1986; Schmidt, 1987; Battaglia & DeSouza, 1989). Da R-MDMA in der vorliegenden Arbeit weniger wirksam im Rotationsverhalten war (II), scheint jedoch die Aktivierung dieser Rezeptoren für die symptomatische Anti-Parkinson-Wirkung nicht essentiell zu sein.

Die Tatsache, dass der selektive Serotonin-Wiederaufnahme-Hemmer Citalopram die Wirkung von MDMA nicht vollständig blockieren konnte (I), verdeutlicht, dass der Membrantransporter nicht das einzige molekulare Ziel von MDMA darstellt, das zur Freisetzung von Serotonin (Mlinar & Corradetti, 2003) und zur symptomatischen Anti-Parkinson-Wirkung von MDMA essentiell ist.

Die Entleerung des serotonergen Systems durch PCPA schwächte ebenfalls die durch MDMA ausgelösten Rotationen ab (I). Hierzu passt auch, dass die Vorbehandlung mit PCPA die durch MDMA induzierte Hyperaktivität abschwächt (Callaway et al., 1990) obwohl dies den akuten Anstieg der extrazellulären Serotoninkonzentration völlig verhinderte (Brodkin et al., 1993).

Jedoch konnte keiner der beiden Einflüsse auf das serotonerge System in der vorliegenden Arbeit die Rotationen, die durch MDMA ausgelöst wurden, komplett verhindern. Daraus

folgt, dass die Serotoninfreisetzung nicht der Schlüssel für das Verständnis der guten symptomatischen Anti-Parkinson-Wirkung von MDMA darstellt.
Die durch MDMA ausgelöste Serotoninfreisetzung kann noch aus einem weiteren Grund nicht (alleine) für die gute symptomatische Anti-Parkinson-Wirkung von MDMA verantwortlich gemacht werden: alle MDMA-Derivate setzen vergleichbare Mengen Serotonin frei (Johnson et al., 1986; Bankson & Cunningham, 2002), aber in ihrer Wirkung auf die Katalepsie unterscheiden sie sich beträchtlich (Schmidt et al., 2002). Ebenso korreliert ihre symptomatische Anti-Parkinson-Wirkung im Rotationsverhalten (I) mit der durch das jeweilige Derivat verursachten Dopaminfreisetzung.

Jedoch gibt es Hinweise, dass die erregenden Eigenschaften von MDMA auf dopaminerge Neuronen der Substantia nigra bei der Ratte durch 5-HT2-Rezeptoren vermittelt werden (Nash, 1990; Yamamoto et al., 1995; Gudelsky et al., 1994). Eine Erhöhung der striatalen Dopaminfreisetzung durch Serotonin wurde ebenfalls beschrieben (Benloucif & Galloway, 1991; Bonhomme et al., 1995; Yadid et al., 1994). Außerdem gibt es Hinweise, dass Serotonin eine erregende Wirkung auf bestimmte nigrostriatale Neuronen ausübt, indem es die GABAergen striatonigralen Afferenzen hemmt (Johnson et al., 1992). Neben dem GABAergen System (Yamamoto et al, 1995; Sprague et al., 1998; Simantov, 2004) wird auch eine Rolle für das glutamaterge System (Farfel et al., 1992; Nash & Yamamoto, 1992; Finnegan & Taraska, 1996) bei der Beeinflussung dopaminerger Neuronen angenommen, das die Wirkung von MDMA vermitteln könnte. Andere Berichte gehen davon aus, dass unter basalen Bedingungen Serotonin keinen Einfluss auf die Dopaminfreisetzung hat, aber die Dopaminfreisetzung positiv beeinflusst, falls die nigrostriatale Dopamintransmission aktiviert ist (Lucas et al., 2000). Es wurde diskutiert, dass serotonerge Terminalen im Striatum L-DOPA zu Dopamin umwandeln können und dann Dopamin anstatt Serotonin freisetzen (Tanaka et al., 1999).

Es wurde festgestellt, das MDMA auch extrazelluläre Spiegel von Noradrenalin und Neuropeptiden erhöht (White et al., 1996). Die Erhöhung der noradrenergen Aktivität hat nur eine geringe Anti-Parkinson-Wirkung (Rückert, 2001), obwohl es einige Hinweise auf neuroprotektive Wirkungen von Noradrenalin bei Morbus Parkinson gibt (Gesi et al., 2000). Außerdem haben elektrophysiologische Untersuchungen gezeigt, dass Noradrenalin als Haupteffekt die Feuerrate nigrostriataler dopaminerger Neuronen hemmt (Collingridge & Davies, 1981). Daher scheint es unwahrscheinlich, dass die gute symptomatische Anti-

Parkinson-Wirkung von MDMA durch das noradrenerge System vermittelt wird. Hierfür spricht auch die unterschiedlich gute symptomatische Anti-Parkinson-Wirkung der beiden Enantiomere von MDMA (II), die sich in ihrer Wirkung auf das noradrenerge System nicht unterscheiden (Schmidt et al., 1987; Steele et al., 1987).

Das in vitro pharmakologische Profil für MDMA weist nur geringe Affinitäten (>500µM) für µ-, δ- und κ-Rezeptoren auf (Battaglia et al., 1988; Battaglia & De Souza, 1989), aber neben den Effekten auf die durch MDMA induzierte Lokomotion bei Mäusen (Compan et al., 2003) beeinflussen Opioide auch die belohnenden Eigenschaften von MDMA (Bilsky et al., 1991; Reid et al., 1996).

In der vorliegenden Arbeit führte der verwendete Opiatantagonist Naloxon selbst zu keinen Verhaltensänderungen, weder im Open Field noch im Rotationsverhalten (III). Im Gegensatz zu Untersuchungsergebnissen bei Mäusen (Compan et al., 2003) fehlt der Einfluss der endogenen Opiate auf das Lokomotionsverhalten nach MDMA-Verabreichung bei Ratten, da Naloxon die durch MDMA induzierte Hyperaktivität nicht beeinflusste (III). Allerdings verminderte Naloxon die durch MDMA ausgelösten Rotationen in unilateral 6-OH-Dopamin lädierten Ratten (III). Dies spricht dafür, dass das Rotationsverhalten nicht einfach mit erhöhter Lokomotion gleichzusetzen ist. Die Ergebnisse aus verschiedenen Verhaltenstests können nicht beliebig ausgetauscht werden. Jedes Modell hat besondere Eigenheiten, die in den Ergebnissen zum Ausdruck kommen. Die lokomotionsstimulierenden Eigenschaften von MDMA bei Ratten werden von einigen Autoren den serotonergen Mechanismen des MDMA zugeschrieben (Callaway et al., 1990, 1991, 1992; Rempel et al., 1993). Demzufolge wird die Lokomotion im Open Field über die Freisetzung von Serotonin moduliert und nicht durch endogene Opiate beeinflusst. Das Rotationsverhalten hingegen wird vermutlich (indirekt) durch Dopamin moduliert und ist daher auch durch Opioide beeinflussbar (III).

Die Neurotransmission durch endogene Opioide ist im Striatum von Parkinsonpatienten mit Dyskinesien und in Tiermodellen der Krankheit erhöht. Allerdings ergeben sich bei der Beeinflussung opioiderger Systeme widersprüchliche Aussagen. Einen positiven Effekt auf die Therapie mit L-DOPA hatte der Opioid-Antagonist Naloxon (Fox et al., 2004), der jedoch die positiven Effekte von MDMA in der vorliegenden Arbeit verminderte (III). Allerdings existieren weitere Berichte über eine positive Wirkung von Opioid-Antagonisten (Fox et al., 2002). Die Aktivierung von µ-Rezeptoren produziert oft widersprüchliche Verhaltensantworten. So verursacht Morphin plötzliche Lokomotionssteigerungen (Babbini & Davis, 1972), führt aber in höheren Dosen zu kataleptischem Verhalten (Turski et al., 1982; De Ryck et al., 1980). Da die endogenen Opiate ihrerseits die dopaminerge Transmission in

den Basalganglien beeinflussen, ist es denkbar, dass die beobachteten Effekte eine indirekte Beeinflussung des dopaminergen Systems darstellen. μ- und δ- Rezeptor-Agonisten erhöhen die extrazellulären Dopaminspiegel in Nucleus accumbens und Striatum und führen zur Lokomotionssteigerung (Spanagel, 1995). Des Weiteren beeinflussen sich GABAerge, sowie serotonerge und dopaminerge Mechanismen vor allem innerhalb der Basalganglien gegenseitig. Es ist möglich, dass endogene Opiate erst bei gestörter dopaminerger Transmission eine wichtigere Rolle spielen, da in intakten Ratten keine Veränderung der MDMA-induzierten Lokomotion nach Behandlung mit Naloxon erfolgte (III). Hieraus kann man schließen, das endogene Opiate eine Rolle bei der symptomatischen Anti-Parkinson-Wirkung von MDMA spielen.

Die Einflüsse einiger Faktoren auf die symptomatische Anti-Parkinson-Wirkung von MDMA und seinen Derivaten und Enantiomeren wurden in dieser Arbeit untersucht. Die Erkenntnisse tragen zum Verständnis der pharmakologischen Mechanismen der symptomatischen Anti-Parkinson-Wirkung von MDMA bei. Der vollständige Wirkmechanismus ist jedoch aufgrund seiner Komplexität immer noch unbekannt.

Nach wie vor ist es denkbar, dass sowohl ein noch nicht identifizierter Faktor hierfür verantwortlich ist, oder aber das noch unbekannte Zusammenspiel mehrerer Faktoren. Die Dopaminfreisetzung als alleinigen ursächlichen Faktor bei den beobachteten guten symptomatischen Anti-Parkinson-Wirkungen von MDMA zu betrachten, ist zum gegenwärtigen Zeitpunkt jedoch nicht gerechtfertigt. Auch wenn im Rotationsverhalten nach unilateraler Läsion des medialen Vorderhirnbündels vornehmlich dopaminerge Effekte gemessen werden, ist dies jedoch kein zwingender Beweis für einen dopaminergen Mechanismus als essentiellen Faktor für die gute symptomatische Anti-Parkinson-Wirkung von MDMA.

Welches die entscheidenden Merkmale des Wirkungsspektrums von MDMA sind und wie man dies für die zukünftige Entwicklung von Anti-Parkinsonmitteln nutzen könnte, bleibt weiterhin eine große Aufgabe für die Forschung.

4 Zusammenfassung

Die gute symptomatische Anti-Parkinson-Wirkung von MDMA wurde in Tiermodellen bei der Ratte nachgewiesen. Die Derivate und Enantiomere von MDMA unterscheiden sich in ihrer Wirksamkeit. Im Rotationsverhalten sind die Substanzen effektiver, die stärker auf das dopaminerge System wirken, wie MDA oder das S(+)-Enantiomer von MDMA. Da die Beeinflussung das serotonergen Systems und des Opioid-Systems die gute Wirksamkeit von MDMA behinderte, sind diese Systeme am Wirkmechanismus beteiligt. Das serotonerge System kann für die positive Anti-Parkinson-Wirkung von MDMA nicht alleine verantwortlich gemacht werden, da anderen bekannten Substanzen, die auf dieses System einwirken, eine Anti-Parkinson-Wirkung fehlt. Auch eine Freisetzung von Noradrenalin und Dopamin kann diesen Effekt von MDMA nicht hinreichend erklären. Dass diese und weitere Systeme ihrerseits wiederum das dopaminerge System beeinflussen ist ebenfalls bekannt. Folglich ist es möglich, dass die Wirkung von MDMA durch die direkte und indirekte Beeinflussung des dopaminergen Systems über mehrere andere Transmittersysteme zustande kommt.

Aus der vorliegenden Arbeit kann geschlossen werden, dass sowohl serotonerge, dopaminerge, als auch opioiderge Systeme an der beobachteten symptomatischen Anti-Parkinson-Wirkung von MDMA beteiligt sind.

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6 Abkürzungen

GABA	Gamma-Aminobuttersäure
5-HT	Serotonin
L-DOPA	L-Dihydroxyphenylalanin, Levodopa
MBDB	N-Methyl-1-(1,3-benzodioxol-5-yl)-2-butananamin
MDA	3,4-Methylendioxyamphetamin
MDE	3,4-Methylendioxy-N-ethylamphetamin
MDMA	3,4-Methylendioxymethamphetamin
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetra-hydropyridin
MPP^+	1-Methyl-4-phenyl-pyridinium
6-OHDA	6-Hydroxydopamin
s.c.	subcutan
XTC	Ecstasy

7 Erklärung zum Eigenanteil an den einzelnen Publikationen

Publikation I Komplette konzeptionelle und inhaltliche Planung; komplette Versuchsvorbereitung, Versuchsdurchführung und Auswertung sowie Verfassen des Manuskripts

Publikation II Komplette konzeptionelle und inhaltliche Planung; komplette Versuchsvorbereitung, Versuchsdurchführung und Auswertung sowie Verfassen des Manuskripts

Publikation III Komplette konzeptionelle und inhaltliche Planung; komplette Versuchsvorbereitung, Versuchsdurchführung und Auswertung sowie Verfassen des Manuskripts

Bei keiner der aufgeführten Publikationen ging der Anteil von Herrn Prof. Dr. Werner J. Schmidt über das im Rahmen eines Betreuungsverhältnisses übliche Maß hinaus.

8 Lebenslauf

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9 Anhang: Publikationen

Is the Ecstasy-induced ipsilateral rotation in 6-OHDA unilaterally lesioned rats dopamine independent?

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Keywords: MDMA, 3,4-Methylenedioxymethamphetamine, apomorphine, rotational behaviour, rat, 6-OHDA, Citalopram, MBDB, MDE, MDA, PCPA, serotonin, dopamine, Parkinson's disease

Summary

MDMA has recently been hypothesized to be effective against the symptoms of Parkinson's disease. Therefore we tested the effects of MDMA-derivatives in the rotational behavioural model. Male Sprague Dawley rats were lesioned unilaterally with 6OHDA at the medial forebrain bundle. MDMA was administered at doses of 2.5, 5.0 and 10.0 mg/kg, its derivatives MBDB and MDE and MDA at 5.0 mg/kg respectively. All substances induced ipsilateral rotations, MDA being the most effective. MDMA induced rotations were attenuated by the selective serotonin reuptake inhibitor Citalopram but were not abolished by pre-treatment with the selective serotonin synthesis inhibitor PCPA (para-chlorophenylalanine). The effects of MDMA can therefore not fully be explained by serotonin release or by dopaminergic activity of the drugs.

Abbreviations: ANOVA analysis of variance, GABA gamma-aminobutyric acid, MBDB N-Methyl-1-(1,3-benzodioxol-5-yl)-2-butananamin. MDA 3,4-Methylenedioxyamphetamine, MDE 3,4-Methylendioxy-N-ethylamphetamine, MDMA 3,4-Methylenedioxymethamphetamine, MFB medial forebrain bundle, 6-OHDA 6-Hydroxydopamine, PCPA para-chlorophenylalanine, PBS phosphate-buffered saline, SEM standard error of the mean.

Introduction

Several lines of evidence point to a unique symptomatic antiparkinsonian efficacy of 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy", "X", "e") (Schmidt et al., 2002). However, MDMA is an illegal and even toxic substance (Ricaurte, 2002) and therefore not suited for widespread use. This study is designed to analyse the mechanisms by which MDMA mediates the antiparkinsonian effect. The elucidation of MDMA ´s mechanism of action could represent a basis for the development of new and safe antiparkinsonian drugs.

Parkinson's disease is a progressive neurodegenerative disorder of the dopaminergic mesotelencephalic system. The currently used standard therapy with the dopamine precursor L-DOPA can not prevent the progression of the disease and induces dyskinesias. Furthermore, dopaminergic agonists may even evoke unwanted hallucinatory side effects. For these reasons there is still a need for new pharmacologically active agents in the therapy of Parkinson's disease.

A standard animal model to simulate hemiparkinsonism is the lesioning of the dopaminergic nigrostriatal system by unilateral injections of 6-hydroxy-dopamine (6-OHDA) into the medial forebrain bundle (MFB). Rotational behaviour is the major behavioural method for measuring dopaminergic activity in the rat brain. Unilateral lesioning of the dopaminergic input into the striatum results in walking in circles because the intact striatum is still functioning. This method was first described by Ungerstedt and Arbuthnott (1970). Since

then, it has often been used for effective preclinical screening of substances that can elevate dopamine levels or activate dopamine receptors: drugs that release dopamine from presynaptic terminals and other stimulants induce ipsilateral rotations; drugs which activate postsynaptic dopamine receptors induce contralateral rotations. Consequently, substances which show such effects could in most cases be used as therapeutics for Parkinson's disease.

MDMA has strong motor stimulant effects in animals (Spanos and Yamamoto, 1989) and has recently been shown to counteract haloperidol induced parkinsonian symptoms in rats (Schmidt et al., 2002). However, the neurochemical basis for this stimulant effect is still not completely understood (see discussion).

However, the illicit recreational use of 3,4-Methylendioxymethamphetamine is very popular (Strote et al., 2002). Ecstasy accounted for 10% of consumed illegal drugs 2001 in Germany (Bundeskriminalamt, Rauschgiftjahresbericht 2001(2002)).
MDMA and its derivatives MDE, MBDB and MDA share a basic amphetamine structure and are suggested to represent a novel class of drugs, named entactogens, because of their effects on human psyche (Nichols, 1986; Nichols et al., 1986).

In this study the unilateral 6-OHDA lesion model was chosen to analyse the mechanisms of action of MDMA: At first, the question was addressed whether MDMA and its derivatives MDE, MBDB and MDA are able to induce circling; the next question concerned the direction of the circling response. Since MDMA induced ipsilateral rotations, a presynaptic mechanism of action was assumed. Thereafter it was tested how the serotonin reuptake inhibitor citalopram and serotonin depletion by PCPA affect MDMA actions.

Material and methods

Animals

Male Sprague-Dawley rats weighing between 220 and 320 g were housed in groups of five or six in a temperature- and humidity controlled environment under a 12/12 hours light-dark cycle. Water was available ad libitum and standard rat food was delivered once daily at 12 g per animal. All experiments were carried out during the light phase and were performed in accordance with international ethical standards and the German Animal-Protection Law and have been approved by the local animal care committee (Tierschutzkommission, Regierungspräsidium Tübingen, ZP5/01).

Drugs

The racemic Ecstasy-derivatives MDA, MDMA, MDE and MBDB were synthesized from Piperonal obtaining the hydrochloride salts, according to the methods of Braun and colleagues (Braun et al., 1980) and dissolved in phosphate-buffered saline (PBS, Sigma Taufkirchen, Germany). All drugs were administered subcutaneously in an injection volume of 1 ml/kg. MDMA HCl was administered at 2.5, 5.0 and 10.0 mg/kg, MBDB, MDA and MDE were administered at 5.0 mg/kg respectively. Apomorphine HCl-solution (Teclapharm, Lüneburg, Germany) was diluted in physiological saline and administered at 0.25 mg/kg subcutaneously. Citalopram-HCl-solution (Lundbeck Hamburg, Germany) was diluted in PBS (Sigma Taufkirchen, Germany) and administered at 10 mg/kg subcutaneously. Para-chlorophenylalanine methyl ester HCl (PCPA) (Sigma Taufkirchen, Germany) was diluted in PBS (Sigma Taufkirchen, Germany) and administered subcutaneously on three consecutive days at a dose of 150 mg/kg.

Medial forebrain bundle unilateral lesion

Rats were anesthetised with Pentobarbital-Natrium (Narcoren®, Merial Hallbergmoos, Germany) 60 mg/kg, i.p. and 6-OHDA HBr (8 µg in 1 µl ascorbic acid 0.01%, both Sigma Taufkirchen, Germany), was injected into the left medial forebrain bundle at 0.1 µl per min. The stereotaxic coordinates were: A = -4.0 mm from the interaural line, L = 1.6 mm from bregma and H = 8.8 mm from the surface of the skull according to a stereotaxic atlas (Paxinos and Watson, 1998). The injection cannula was left in place for additional 4 minutes to allow diffusion of the neurotoxin. 30 min prior to the lesion, desipramine HCl (Sigma Taufkirchen, Germany) was administered i.p. at a dose of 20 mg/kg to protect noradrenergic neurons against damage by 6-OHDA. Atropinesulfate (0.2 mg per animal in 0.2 ml saline, Sigma Taufkirchen, Germany) was administered subcutaneously to ease breathing during the anaesthesia. To allow recovery from surgery, animals were singly housed for one day after the surgery.

Behavioural testing

All compounds were tested in the same group of animals, in experiments lasting 120 to 220 min with a wash-out period of at least 5 days between each treatment. Animals were placed into plastic cylinders (23 cm in diameter) and rotational behaviour (360 degree turns) was scored in 10 min intervals. Rotations in the ipsilateral and contralateral directions were counted separately and the analyses were based on the net scores (ipsilateral minus contralateral rotations); a positive score indicated that the animals exhibited a net ipsilateral bias, whereas a negative score indicated a net contralateral bias. Animals were placed into the cylinders 15 min prior to injection of substances and baseline spontaneous rotations were measured over 10 min prior to injection. Depletion of brain serotonin was induced by repeated administration of the serotonin synthesis inhibitor para-chlorophenylalanine methyl ester HCl (PCPA) once daily on three consecutive days at 150 mg/kg. This regimen reportedly leads to a large and selective depletion of 5-HT (Koe and Weissman, 1966). Behavioural testing with MDMA was redone three days afterwards.

Statistical analysis

Data are given as means ± SEM and a p-value of 0.05 was accepted as significant.
Data for Apomorphine and Saline were analysed by one-way analysis of variance (ANOVA) followed by Dunnett´s test for comparison with baseline. For comparison of several treatments the results were analysed by two-way ANOVA with repeated measures followed by Tukey/Kramer test for multiple pairwise comparisons.

Results

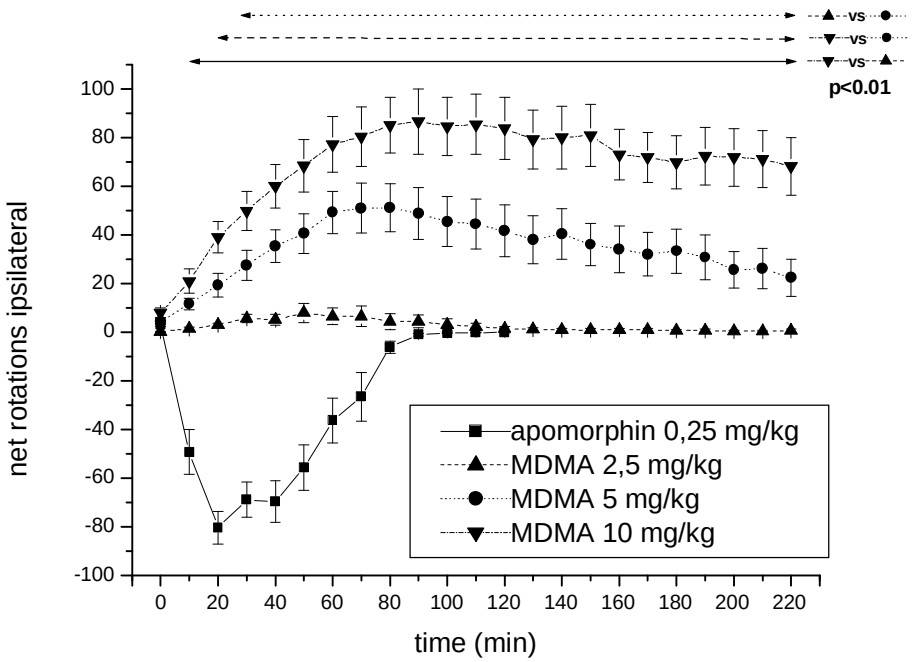

Fig. 1: Time course of rotations after administration of apomorphine and of MDMA in 6-OHDA MFB lesioned rats (means ± S.E.M., n=11). Apomorphine induced rotations to the side contralateral to the lesion [F (12,120) =34,50, p<0.0001]. MDMA caused dose-dependent rotations to the side ipsilateral to the lesion [F (44,660) =10.03, p<0.0001]. Lines with arrows indicate p<0.01 versus respective group.

Apomorphine induced contralateral rotations [F(12,120) =34,50, p<0.0001] beginning immediately after the injection and lasting approximately 80 minutes with a peak reaction 20 minutes after the injection (Figure 1).

Injection of MDMA (5 mg/kg and 10 mg/kg) induced dose dependent rotations [F(44,660) =10.03, p<0.0001] in ipsilateral direction to the lesion of the MFB (Figure 1). The administration of 2.5 mg/kg MDMA did not produce a significant effect from baseline; higher dosages of MDMA produced rotations with a peak reaction at 70 respectively 90 minutes after the injection. The rotations did not reach baseline levels by the end of registration of

rotations after 220 minutes in the higher dosage. Time course of rotations after administration of MDMA and its derivatives revealed significant differences [F(66,880) =6,59, p<0.0001].

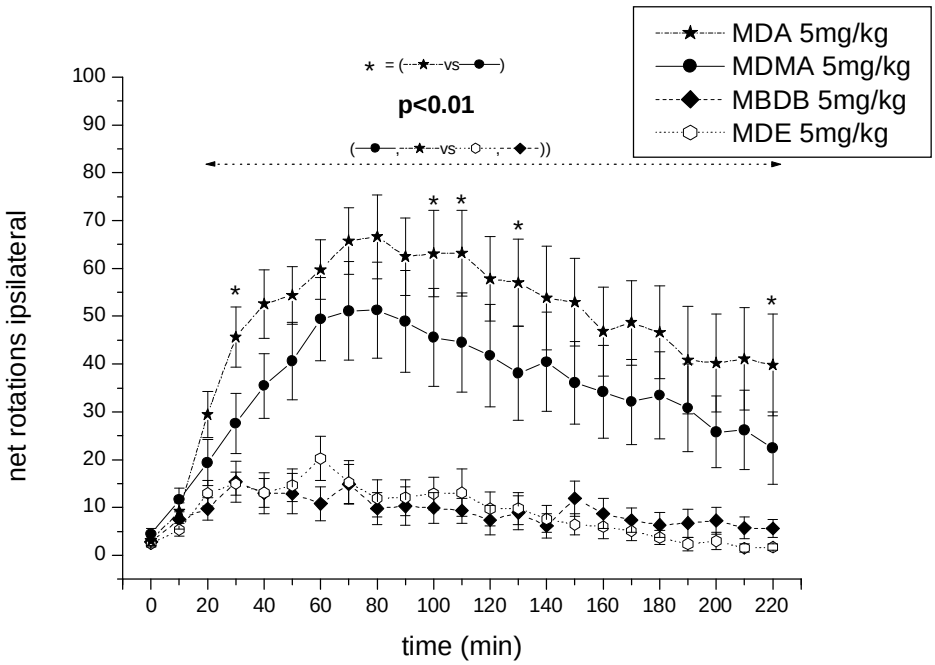

Fig. 2: Time course of rotations after administration of MDMA and its derivatives (5 mg/kg respectively) in 6-OHDA MFB lesioned rats (means ± S.E.M., n=11) [F (66,880) =6,59, p<0.0001]. MDA is most potent at inducing ipsilateral rotations, tightly followed by MDMA, while MDE and MBDB having no detectable effect except for MDE at 60 minutes. Lines with arrows indicate p<0.01 versus respective group.

Both MBDB and MDE at 5 mg/kg did not induce significant ipsilateral rotations from baseline except for MDE after 60 minutes. There was a slight tendency for rotations but MBDB and MDE were far less potent than MDMA at the same dosage (Figure 2). The number of net rotations induced by MBDB did not differ at any point from MDE in the time course of this study. MDA was even more potent than MDMA with the net number of ipsilateral rotations peaking at 80 minutes (Figure 2). The time course of rotations after MDA was similar to that of MDMA at the same dosage.

The selective serotonin reuptake inhibitor citalopram (10 mg/kg) markedly attenuated MDMA- (5 mg/kg) induced rotations in combined treatment while citalopram administration itself had no effect [F(44,660) =9,11, p<0.0001], (Figure 3). The time course of the reaction in combined treatment was also delayed with a peak effect only seen after 160 minutes.

After a serotonin depleting regimen of PCPA, MDMA induced rotations were slightly reduced [F(44,660) =8,53, p<0.0001] (Figure 4). The time course of MDMA induced rotations was also delayed after PCPA treatment with a peak reaction at 130 minutes in comparison to a peak reaction at 70 minutes before PCPA administration. Acute effects of PCPA administration on rotational behaviour were also registered at the first time of PCPA administration but no significant effect could be observed (Figure 4).

After Saline-administration slight ipsilateral rotations were observed [F(17,170) =2,01, p<0.013].

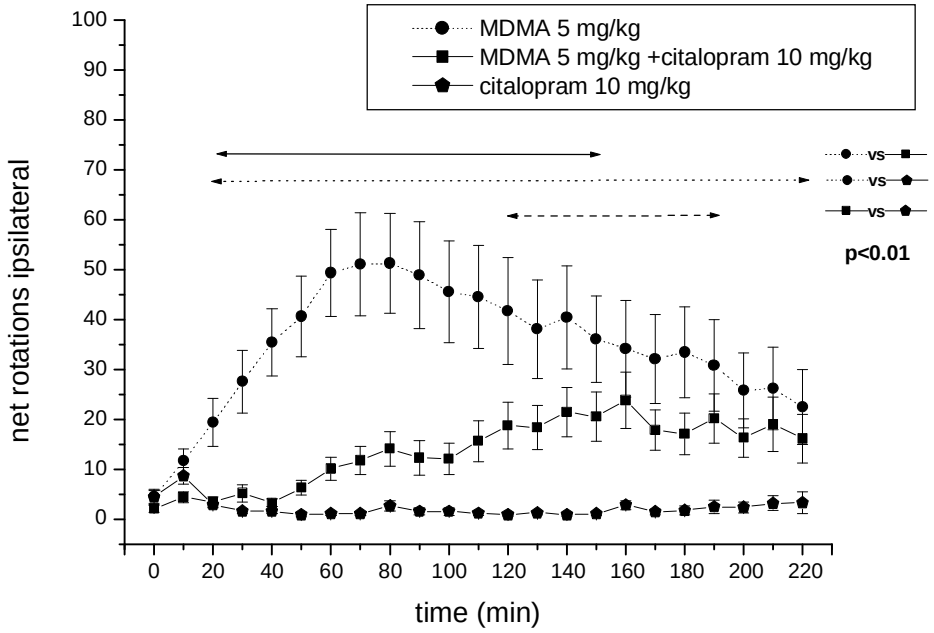

Fig. 3: Effect of citalopram (10mg/kg) administration on MDMA (5 mg/kg)-induced rotations in 6-OHDA MFB lesioned rats (means ± S.E.M., n=11) [F(44,660) =9,11, p<0.0001]. Citalopram attenuated and delayed rotations in combined treatment with MDMA while having no effect on its own. Lines with arrows indicate p<0.01 versus respective group.

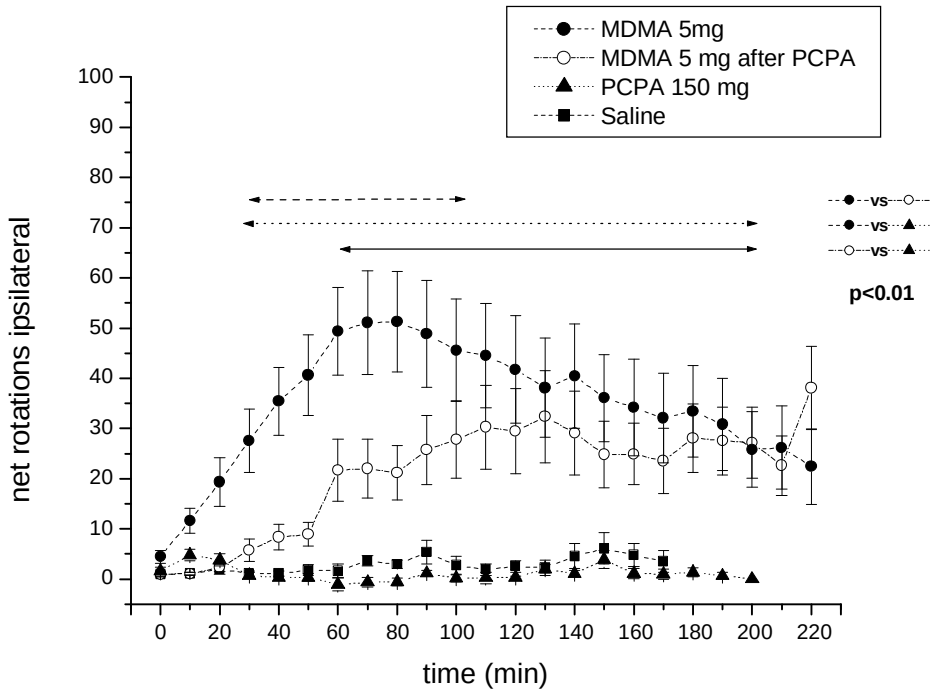

Fig.4: Effect of PCPA pretreatment on MDMA (5 mg/kg)-induced rotations [F(44,660) =8,53, p<0.0001] and conditioned effect of Saline treatment [F(17,170) =2,01, p<0.013] after repeated testing in 6-OHDA MFB lesioned rats (means ± S.E.M., n=11)]. Serotonin depletion by treatment with PCPA attenuated and delayed the effect of MDMA on rotational behaviour while having no acute effect on its own. After repeated testing with several drugs, the animals rotated after administration of Saline. Lines with arrows indicate p<0.01 versus respective group.

Discussion

Circling after administration of apomorphine was tested as proof of the lesion because several studies have shown that only animals with a complete lesion rotate to the contralateral side following apomorphine administration (Hefti et al., 1980; Hudson et al., 1993; Barnéoud et al., 1995), although there are reports on rotations in partially lesioned animals (Przedborski et al., 1995).

MDMA caused ipsilateral rotations in a dose-dependent and reproducible manner. The strength of this ipsilateral rotational behaviour increased within the following order of the MDMA-derivatives: MBDB, MDE, MDMA, MDA.

Citalopram attenuated MDMA induced rotations but did not induce rotations if administered alone. Serotonin depletion by PCPA also slightly attenuated MDMA-induced rotations but did not completely abolish rotations. Saline-administration after all treatments revealed a small effect of rotation to the ipsilateral side of the lesion.

The slight rotational behaviour after saline injection is interpreted as conditioned effect caused by repeated testing. Conditioned effects of repeated testing in rats with unilateral 6-hydroxydopamine lesions have been described before (Carey 1986a, 1986b, 1988).

The rotational behaviour test is one of the most powerful tools to screen potential anti-Parkinsonian agents. It bases on the finding that the level of dopamine in the substantia nigra is very low in Parkinson's patients and in MFB 6-hydroxydopamine lesioned animals. Potential anti-Parkinsonian agents can either stimulate dopamine release from presynaptic terminals or they can activate postsynaptic dopamine receptors. In order to verify the lesion, apomorphine was administered and animals were expected to rotate contralaterally to the side of lesion. All animals in the current study fulfilled this criterion and were therefore considered hemiparkinsonian.

For the induction of contralateral rotations, postsynaptic receptor specifity of the respective drugs is required. In contrast, ipsilateral rotations can not be considered to be specific for a direct dopamine releasing action because there are many actions in the nervous system that indirectly induce presynaptic dopamine release from nigrostriatal neurons. A variety of drugs that influence cholinergic, noradrenergic, GABAergic, opioid and substance-P systems of the brain can induce turning responses, when investigated either following lateralized intracerebral administration or given peripherally to animals with a variety of unilateral central lesions (Pycock, 1993). Because in our experiments MDMA induced ipsilateral rotations, primarily presynaptic mechanisms are discussed.

The direction of rotations after administration of MDMA was the same as after injection of amphetamine which is thought to mediate this effect by releasing dopamine from presynaptic terminals. Predominantly amphetamine-like rotation has also been observed in unlesioned animals after administration of MDMA (Kulmala et al., 1987). Amphetamine shares some behavioural properties with MDMA but the two substances also differ in other behavioural and neurochemical effects and MDMA is therefore thought to represent a class of drug different from psychomotor stimulants (Nichols et al., 1986).

<u>Does dopamine account for the antiparkinsonian effects of MDMA?</u>

It can not be ignored that MDMA, like amphetamine, releases dopamine (Nash, 1990; Nash & Nichols, 1991; McKenna et al., 1991; Colado & Green,1994; White et al. 1996; Kankaanpaa et al., 1998; Bankson & Cunningham, 2001). A role for the mesolimbic dopamine system in the psychostimulant actions of MDMA has been discussed since DA depletion with the DA neurotoxin 6-OHDA attenuated the behavioural effects of MDMA (Gold et al., 1989).

The potency to release striatal dopamine decreases in the following order: MDA, MDMA, MDE, MBDB (Nash & Nichols, 1991; Johnson et al., 1986). In our experiments, the

same order was seen in the extent of rotational behaviour. The time course of rotations after MDMA and MDA was very similar though MDA being more effective. This suggests that both substances are inducing rotations by similar mechanism. But it can not be concluded from these reports that the effects of MDMA and its derivatives in the current study are only due to dopamine release.

It seems rather unlikely that direct dopamine release mediates ipsilateral rotations, since combined treatment with Citalopram markedly reduced the number of MDMA-induced rotations. It has been shown that MDMA-induced serotonin release is inhibited by serotonin reuptake inhibitors but dopamine release is not altered (Mechan et al., 2002). It is assumed that blockade of the serotonintransporter with citalopram prevents MDMA from entering serotonergic neurons leaving dopamine neurons unaffected. Thus, the MDMA-effect seems to be mediated by serotonergic neurons but not by dopamine neurons. In conclusion, it seems rather unlikely that MDMA acts directly upon dopamine neurons to release dopamine.

Does serotonin account for the antiparkinsonian effects of MDMA?

It has been shown that MDMA releases serotonin and norepinephrine more potently than dopamine (Rothman et al. 2001). However, in general, an elevated serotonin level is not associated with reduced parkinsonism; on the contrary, serotonin release or reuptake inhibition rather enhances parkinsonian symptoms in the catalepsy model in rats (Wadenberg, 1996) and there have also been reports of de novo onset of parkinsonian symptoms in humans (Stadtland et al., 2000). These effects are thought to be caused by inhibition of dopaminergic neurons by serotonergic mechanisms (Kelland et al, 1990; Neal-Beliveau et al., 1993).

From the attenuation of MDMA induced rotations by Citalopram, it can be concluded that the effect of MDMA on rotational behaviour is at least in part mediated via uptake into serotonergic neurons. But it is rather unlikely, that the rotations are elicited by a simple blockade of reuptake and enhanced extracellular levels of serotonin like it would be mediated by serotonin reuptake inhibitors, for Citalopram in the current study did not induce rotations by itself. In contrast to MDMA, Citalopram is reported not to influence dopamine and norepinephrine but only to increase serotonin levels (Millan et al., 2000). Findings in other studies with Citalopram also suggest that physiological effects of MDMA in humans are partially due to an interaction of MDMA with the serotonin carrier and a subsequent release of serotonin (Liechti & Vollenweider, 2000). In conclusion it appears unlikely that enhanced serotonin levels account for the antiparkinsonian effects of MDMA.

Nevertheless, pharmacological data from human studies showing that some of the behavioural effects of MDMA (positive mood, extroversion, heightened sensory perception) are blocked by the SSRI Citalopram (Liechti et al., 2000) support the involvement of serotonin transporters in the mechanism of action of ecstasy. However, it has also been suggested that the excitatory effects of MDMA on dopaminergic neurons of the rat substantia nigra are mediated by 5-HT_2 receptors (Nash, 1990; Yamamoto et al., 1995; Gudelsky et al., 1994). Enhancement of striatal dopamine release by serotonin has been reported (Benloucif & Galloway, 1991; Bonhomme et al., 1995; Yadid et al., 1994). There is also evidence for MDMA inhibiting the dopamine reuptake carrier (Metzger et al., 1998). In addition, there is evidence that serotonin can mediate an excitatory action on a subset of nigrostriatal neurons by inhibiting striatonigral GABAergic afferents (Johnson et al., 1992). Other findings indicate that, in the striatum, endogenous serotonin has no influence on dopamine release under basal conditions, but positively modulates dopamine outflow when nigro-striatal DA transmission is activated (Lucas et al., 2000). Tanaka et al. (1999) have suggested that serotonergic terminals in the striatum can convert administered L-DOPA into dopamine exogenously and in the absence of dopaminergic terminals. Dopamine would be released instead of serotonin and drugs acting to stabilize serotonergic neurons could help to prevent side effects of L-

DOPA. Therefore, it seems possible that MDMA induces rotations in part via one of these mechanisms.

It has already been assumed that MDMA-induced serotonin release can not fully account for all the positive effects of MDMA in animal models of Parkinson's disease for another reason: all derivatives of MDMA are equipotent releasers of serotonin but their effects on catalepsy differ considerably (Schmidt et al., 2002; Bankson & Cunningham, 2002). Additionally, our experiments yield another evidence that serotonin can not be the key for understanding the antiparkinsonian effect of MDMA: serotonin depletion with PCPA failed to block rotations induced by MDMA in our experiments though it has been shown that pre-treatment with PCPA attenuated (+)-MDMA induced hyperactivity (Callaway et al., 1990).

Repeated treatment with PCPA reportedly leads to a large and selective depletion of serotonin in vitro and in vivo (Koe & Weissman, 1966). Biochemical reports have indicated that the serotonin levels are recovering in 8 - 15 days after p-chlorophenylalanine treatment probably involving an upregulation of tryptophanhydroxylase gene expression (Park et al., 1994). But there are also differences in types of serotonin neurons differentially responding to treatment with p-chlorophenylalanine (Tohyama et al., 1988). The tryptophan hydroxylase inhibitor PCPA has been reported to block the acute increase in the extracellular concentrations of serotonin produced by MDMA completely, but although PCPA significantly attenuated the increase in dopamine efflux produced by MDMA, the effect was small in magnitude (Brodkin et al., 1993). Therefore it can be assumed that serotonin can not account for MDMA induced rotations alone, but it could contribute to the observed effects.

Are there other explanations?

MDMA has also been found to increase extracellular levels of norepinephrine and to alter brain levels of neuropeptides (White et al., 1996). Enhancing norepinephrine activity has shown only some weak antiparkinsonian activity (Rückert, 2001), although there are several hints of neuroprotective actions of norepinephrine in Parkinson's disease (Gesi et al., 2000). Moreover, electrophysiological studies have shown that the main effect of norepinephrine is to inhibit the firing rate of nigrostriatal dopaminergic neurons (Collingridge and Davies, 1981). For these reasons it seems unlikely that the observed effects of MDMA are simply due to its action on the norepinephrine system.

In regard of the brain dopaminergic, serotonergic and noradrenergic systems, no system can alone be held responsible or excluded to mediate the effects of MDMA observed in this study. It is even possible, that a unique combination of the effects of MDMA on all these systems is responsible for the ipsilateral rotations observed in the current study. It seems also possible that a so far unconsidered mechanism is accountable for these effects.

In conclusion, so far we can not convincingly explain MDMA´s mechanism of action. Its efficacy in the rotational model of hemiparkinsonism is critically dependent on the uptake into serotonin neurons, but seems not to be mediated (solely) by serotonin. Dopamine is also not (only) responsible for these effects. It is concluded that either a combination of the effects on various neurotransmitter systems is responsible for the anti-Parkinsonian effect of MDMA or a so far unknown mechanism of MDMA results in these positive effects.

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3,4-Methylenedioxymethamphetamine counteracts akinesia enantioselectively in rat rotational behaviour and catalepsy

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Keywords: MDMA, 6-OHDA, enantiomers, serotonin, dopamine, Parkinson's disease

Abstract

We have shown recently that 3,4-Methylenedioxymethamphetamine (MDMA) has symptomatic antiparkinsonian activity in rodent models of Parkinson´s disease.
In search of its mechanism of action, we further investigated the enantiomers of MDMA in the rotational behavioural model and catalepsy test. Catalepsy testing was done in drug naïve unlesioned animals. The parkinsonian symptoms rigor and akinesia (i.e. catalepsy) were induced by intraperitoneal administration of haloperidol 0,5 mg / kg and were measured repeatedly as descent latency from a horizontal bar and a vertical grid. MDMA and both its enantiomers were effective in counteracting haloperidol-induced catalepsy, but if given as racemic, the effects were more pronounced than with the enantiomers.
For testing of rotational behaviour, male Sprague Dawley rats were lesioned unilaterally with 6-hydroxydopamine at the medial forebrain bundle. Administration of S-MDMA (5 mg / kg) produced ipsilateral rotations. R-MDMA was far less effective in inducing ipsilateral rotations in 6-hydroxydopamine unilaterally lesioned rats but when S-MDMA was given additionally, rotations immediately increased.
Conclusion: Regarding their overall antiparkinsonian effects, the S-enantiomer of MDMA was more effective than its R-congener. R-MDMA was able to increase the actions of S-MDMA.

Introduction

3,4-Methylenedioxymethamphetamine (MDMA), better known as "Ecstasy", is a ring-substituted amphetamine analogue (Shulgin & Nichols, 1978) belonging to a class of drugs known as entactogens (Nichols, 1986). It is an indirect monoaminergic agonist and monoamine reuptake inhibitor influencing serotonin, dopamine, noradrenaline, acetylcholine and histamine (Green et al., 1995; McDowell & Kleber, 1994; Fischer et al., 2000).
With some selectivity, MDMA binds to serotonin transporters, blocks serotonin reuptake and enhances serotonin release (Nichols et al., 1982; Rudnick & Wall, 1992; Gudelsky & Nash, 1996). The enhanced release of serotonin activates several types of serotonin receptors, but the molecular steps resulting from these effects are still not fully understood. Further complicating is the influence of MDMA on dopaminergic neurons, which is in part mediated by serotonergic (Brodkin et al.,1993; Gudelsky et al., 1994; Gudelsky & Nash, 1996; Shankaran & Gudelsky, 1998; Yamamoto et al., 1995), GABAergic (Yamamoto et al., 1995; Sprague et al., 1998; Simantov, 2004) and glutamatergic systems (Farfel et al., 1992; Nash & Yamamoto, 1992; Finnegan & Taraska, 1996).
We have reported on the anticataleptic and rotational behavioural-inducing properties of MDMA in unilaterally 6-hydroxydopamine lesioned rats before (Schmidt et al., 2002,

Lebsanft et al., 2003) and there has been a report on a patient with Parkinson´s disease who effectively cured symptoms of the disease with tablets of ecstasy (Margolis, 2001). In the former study (Schmidt et al., 2002) we observed differences in the anticataleptic efficacy of the enantiomers of different MDMA derivatives. The current study was designed to compare the anti-akinetic potencies of the enantiomers of MDMA in two animal models of Parkinson´s disease, i.e. the catalepsy test and rotational behaviour.

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles River, Germany) were acclimatized and handled by the experimenter for about one week before start of the experiments. The rats were housed in groups of six in a temperature- (23°C room temperature) and humidity- (55%) controlled environment under a 12/12 hours light-dark cycle. Water was available ad libitum and standard rat food was delivered once daily at 12 g per animal. All experiments were carried out during the light phase and were performed in accordance with international ethical standards and the German Animal-Protection Law and have been approved by the local animal care committee (Tierschutzkommission, Regierungspräsidium Tübingen, ZP 05/01).

Drugs

All drugs were freshly dissolved on the day of testing and administered subcutaneously respectively intraperitoneally in an injection volume of 1 ml/kg. Racemic MDMA HCl, R-MDMA HCl and S-MDMA HCl were kindly supplied by the Pharmaceutical Institute, Department of Pharmaceutical Chemistry/Analysis, University of Tuebingen, Germany and dissolved in phosphate-buffered saline (PBS, Sigma, Taufkirchen, Germany). Haloperidol (Haldol®-Janssen, Janssen Pharmaceutica N.V., Beerse, Belgium) was dissolved in saline and administered at 0,5 mg/kg respectively.

Catalepsy testing

In order to induce catalepsy, rats were treated with haloperidol 0,5mg/kg intraperitoneally. Thirty minutes later, the first injection of the test substance (vehicle (n=10), R-MDMA (2,5 mg/kg, n=10), S-MDMA (2,5 mg/kg, n=10), racemic MDMA (2,5 mg/kg, n=10)) was given subcutaneously followed by the injection of the respective other test substance another 42 minutes later. During that time, animals were kept in their home cages in the experimental room. Catalepsy testing was done 30 minutes after the first and 30 minutes after the second subcutaneous injection of the respective test substance. Catalepsy consisting of the symptoms akinesia and rigidity was measured in two established tests in the following order: bar (both forepaws were placed on a horizontal bar 9 cm above the ground), grid (the animal was hung on a vertical grid with all paws). The degree of catalepsy was quantified by measuring the descent latency represented by the time from placement of the animal until the active movement of one of its paws with a cut-off time of 180 seconds. This procedure was repeated once to avoid novelty effects when animals are placed for the first time on the bar or grid.

Rotational behaviour

After acclimatization rats were lesioned by 6-hydroxydopamine infusion into the medial forebrain bundle (MFB):

Rats were anesthetized with Pentobarbital-Natrium (Narcoren®, Merial Hallbergmoos, Germany) 60mg/kg, i.p. and 6-OHDA HBr (8µg in 1µl ascorbic acid 0.01%, both Sigma Taufkirchen, Germany), was injected into the left medial forebrain bundle at 0.1µl per min. The stereotaxic coordinates were: A = -4.0 mm from the interaural line, L = 1.6mm from bregma and H = 8.8 mm from the surface of the skull according to a stereotaxic atlas (Paxinos & Watson, 1998). The injection cannula was left in place for additional 4 minutes to allow diffusion of the neurotoxin. 30min prior to the lesion, desipramine HCl (Sigma Taufkirchen, Germany) was administered i.p. at a dose of 20mg/kg to protect noradrenergic neurons against damage by 6-hydroxydopamine. Atropinesulfate (0.2mg per animal in 0.2ml saline, Sigma Taufkirchen, Germany) was administered subcutaneously to ease breathing during the anaesthesia. To allow recovery from surgery, animals were singly housed for one day after the surgery. The retrograde degeneration of the dopaminergic cells originating in the substantia nigra was allowed to fully develop during the next 29 days. During that period, the rats were kept in their home cages.

Animals were placed into the plastic bowls of a rotameter system (TSE, Technical & Scientific Equipment GmbH, 61350 Bad Homburg, Germany) and baseline rotational behaviour (360 degree turns) was recorded over a time span of 10 minutes. Immediately afterwards, rat were injected subcutaneously with R-MDMA (5 mg/kg, n=12) respectively S-MDMA (5 mg/kg, n=12) and rotational behaviour was recorded for 60 minutes. Afterwards, the respective other enantiomer of MDMA was injected, the animals were kept in the apparatus and rotational behaviour was recorded for further 60minutes. This procedure was done on two days with a wash-out-period of 18 days and with reversal of treatment group on the second treatment day so that the group that had received first R-MDMA and one hour later S-MDMA on the first day, received first S-MDMA and one hour later R-MDMA on the second day. Half of each treatment group was injected once with racemic MDMA (5 mg/kg) four days after the second treatment with the enantiomers of MDMA. Rotational behaviour was recorded for 120 minutes after 10 minutes of baseline recording before drug administration.
Rotations in the ipsilateral and contralateral (clockwise) directions were counted separately and the analyses were based on the net scores; a positive score indicated that the animals exhibited a net ipsilateral bias. Data were recorded by the TSE rotameter software as *.dat file and stored as *txt in ASCII format. Export files*.csv compatible to *.xls (Microsoft EXCEL) were generated.

Statistical analysis
Statistical analysis was performed by ANOVA followed by post-hoc tests using GB STAT version 7.0. Data are given as means ± SEM and a p-value of 0.05 was accepted as significant.
For catalepsy experiments, descent latencies were analysed using non-parametrical Kruskal-Wallis one-way analysis of variance (ANOVA) since data of catalepsy tests do not follow a normal distribution. Post hoc comparisons with Newman-Keuls test for multiple comparisons were used to detect significant differences between descent latencies of groups.
For rotational behaviour of the enantiomers of MDMA, the net rotations per 10 minutes were analysed by two-way ANOVA with repeated measures followed by post-hoc Tukey/Kramer Procedure t-Test for multiple pair wise comparisons. For rotational behaviour of the racemic MDMA, the net rotations per 10 minutes were analysed by one-way ANOVA with repeated measures followed by post-hoc Tukey/Kramer Procedure t-Test for multiple pair wise comparisons.

Results

Catalepsy testing

Catalepsy induced by haloperidol (0,5 mg/kg) and measured as increases in descent latency from a horizontal bar and vertical grid was counteracted by racemic MDMA (2,5 mg/kg) and both enantiomers of MDMA (2,5 mg/kg) in the first bar [χ^2= 17.456 , p=0. 0006] and grid test [χ^2= 13.649 , p=0. 0034] (Figure 1). In the second bar [χ^2= 11.642 , p=0. 0087] and grid test [χ^2= 14.545 , p=0. 0022], descent latency further increased in haloperidol treated animals but racemic MDMA and S-MDMA counteracted this, R-MDMA was less effective in blocking catalepsy induced by haloperidol. After administration of the respective other enantiomer or racemic MDMA as third injection, all MDMA treatments blocked haloperidol-induced catalepsy in the third bar [χ^2= 14.951 , p=0. 0019] and grid test [χ^2= 16.033 , p=0. 0011]. In the fourth bar [χ^2= 14.600 , p=0. 0022] and grid test [χ^2= 14.921 , p=0. 0019], descent latency in haloperidol treated animals again increased, especially as measured in the bar test where all MDMA treatments were counteracting catalepsy. In the fourth grid test, there was a slight increase in descent latency of animals treated with R- and S-MDMA.

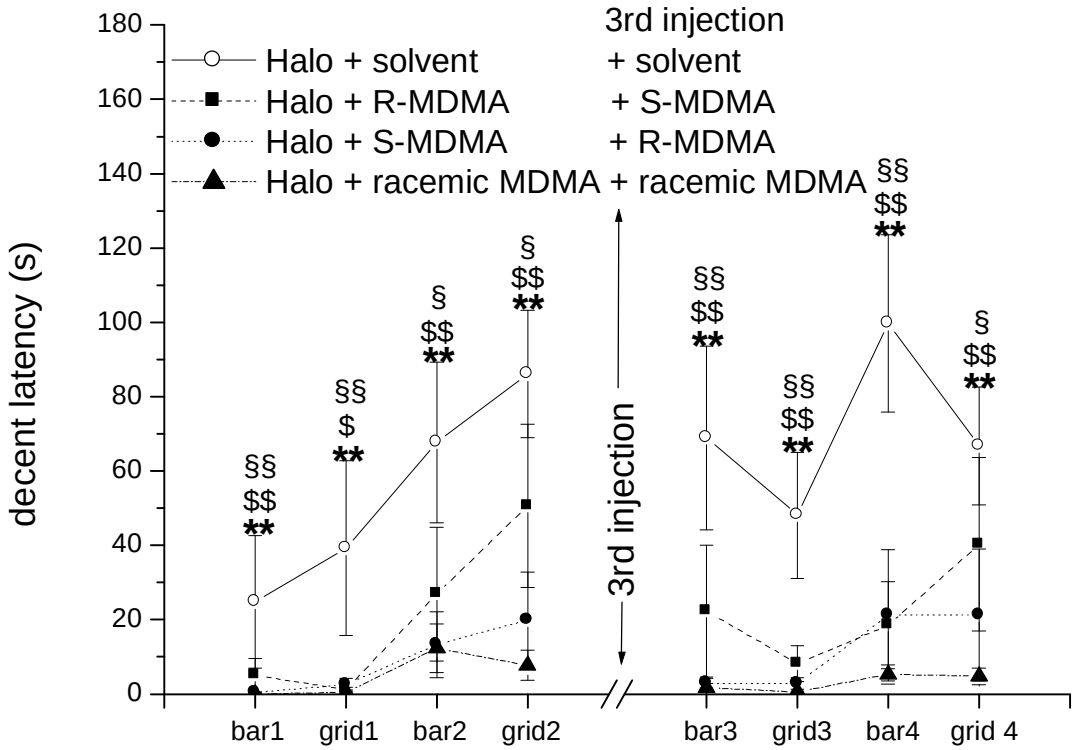

FIG. 1. MDMA decreased descent latency in rats treated with haloperidol 0.5 mg/kg as first injection and the respective drugs (n=10 per group) as second (2,5 mg/kg) and third injection (2,5 mg/kg). Data are shown as mean ± SEM. ** $p<0.01$ compared to group ▲(haloperidol + racemic MDMA + racemic MDMA), $ $p<0.05$ compared to ● (haloperidol + S-MDMA + R-MDMA),$$ $p<0.01$compared to ● (haloperidol + S-MDMA + R-MDMA), § $p<0.05$ compared to ■ (haloperidol + R-MDMA + S-MDMA), §§ $p<0.01$compared to ■ (haloperidol + R-MDMA + S-MDMA). Data were analysed by Kruskal-Wallis One-Way ANOVA followed by post hoc Newman-Keuls test for multiple comparisons.

Rotational behaviour

Injection of S-MDMA (5 mg/kg) induced rotations [$F(1,22) = 9.17614$, $p=0.0062$] in ipsilateral direction to the side of the lesion of the MFB (Figure 2). Rotations increased over time [$F(5,110) = 10.28803$, $p<0.0001$]. The rotations did not reach baseline levels by the end of registration of rotations.

After injection of the respective other enantiomer (Figure 2), a statistical analysis of variances revealed no difference between treatments any more [$F(1,22) = 0.52234$, $p=0.4775$] though a significant effect of time course still was evident [$F(5,110) = 4.66399$, $p=0.0007$].

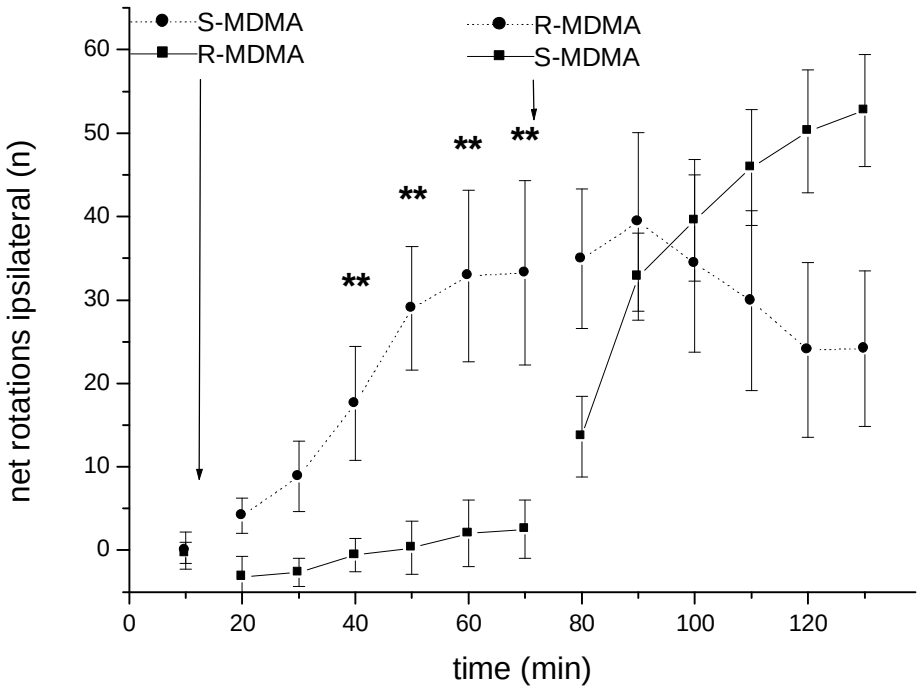

FIG. 2. Time course of rotations of unilaterally 6-OHDA-lesioned rats on the first day of treatment with enantiomers of MDMA (n=12 per group). After 10 minutes of baseline recording animals received the first injection of MDMA (5 mg/kg) followed by the respective other enantiomer (5 mg/kg) 60 minutes later. Data are shown as mean ±SEM. **p<0.01 vs ■ (R-MDMA) at the respective time point. Net rotations per 10 minutes were analysed by two-way ANOVA with repeated measures followed by post-hoc Tukey/Kramer Procedure t-Test for multiple pair wise comparisons.

On the second day the treatment of the animals from day one was reversed from injection R-S to S-R. Injection of S-MDMA (5 mg/kg) to animals that had received first R-MDMA and later S- MDMA the day before, again induced dose dependent rotations [$F(1,22) = 19.81785$, $p=0.0002$] in ipsilateral direction to the side of the lesion of the MFB (Figure 3). There was an increase of rotations over time [$F(5,110) = 11.50271$, $p<0.0001$] like the day before. The rotations also did not reach baseline levels by the end of registration of rotations.

After injection of the respective other enantiomer on the second day (Figure 3), a statistical analysis of variances revealed no effect of treatment any more [$F(1,22) = 0.87558$, $p=0.3596$] though an effect of time course still was evident [$F(5,110) = 10.20752$, $p<0.0001$] like the day before.

In contrast to the first day of treatment, additional treatment with R-MDMA after S-MDMA resulted in prolonged and further increased rotations ipsilateral to the side of the lesion.

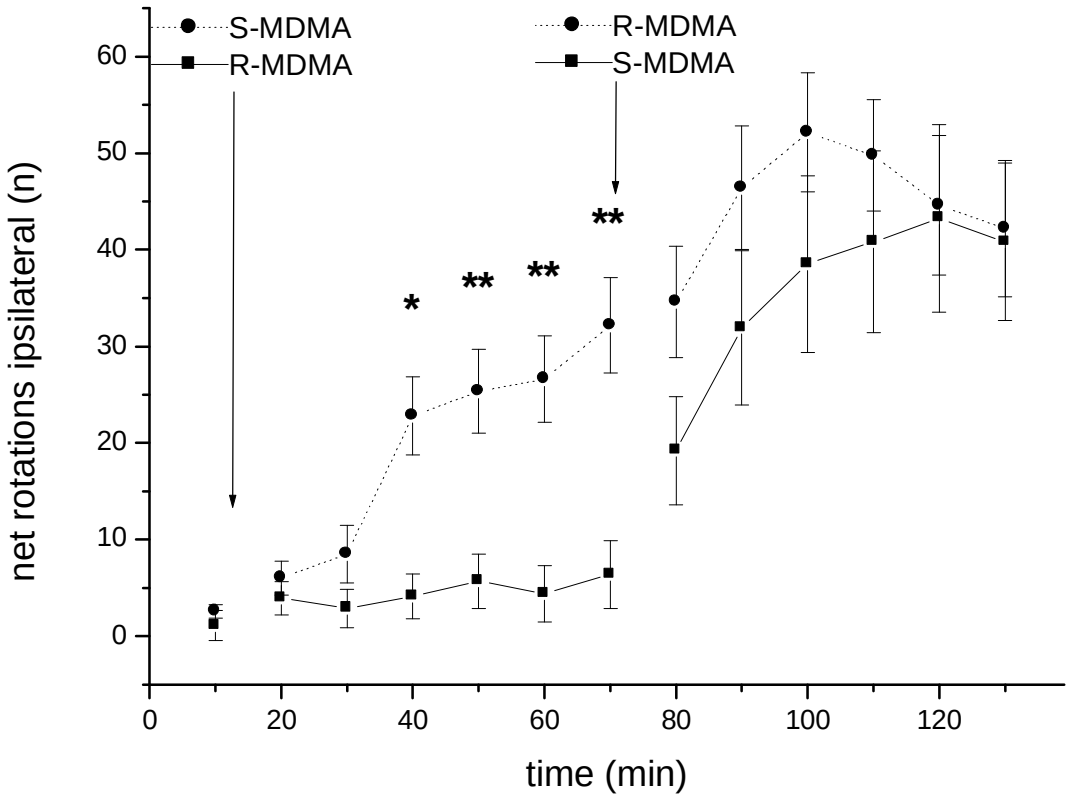

FIG. 3. Time course of rotations of unilaterally 6-OHDA-lesioned rats on the second day of treatment with enantiomers of MDMA (n=12 per group). After 10 minutes of baseline recording animals received the first injection of MDMA (5 mg/kg) followed by the respective other enantiomer (5 mg/kg) 60 minutes later. Treatment groups were reversed compared to day 1. Data are shown as mean ±SEM. **p<0.01 vs ■ (R-MDMA) at the respective time point. Net rotations per 10 minutes were analysed by two-way ANOVA with repeated measures followed by post-hoc Tukey/Kramer Procedure t-Test for multiple pair wise comparisons.

Injection of racemic (R/S-)-MDMA (5 mg/kg) to animals pretreated with the enantiomers of MDMA induced rotations [$F(12,132) = 11.6598$, $p<0.0001$] in ipsilateral direction to the lesion of the MFB (Figure 4). The rotations did not reach baseline levels by the end of registration period.

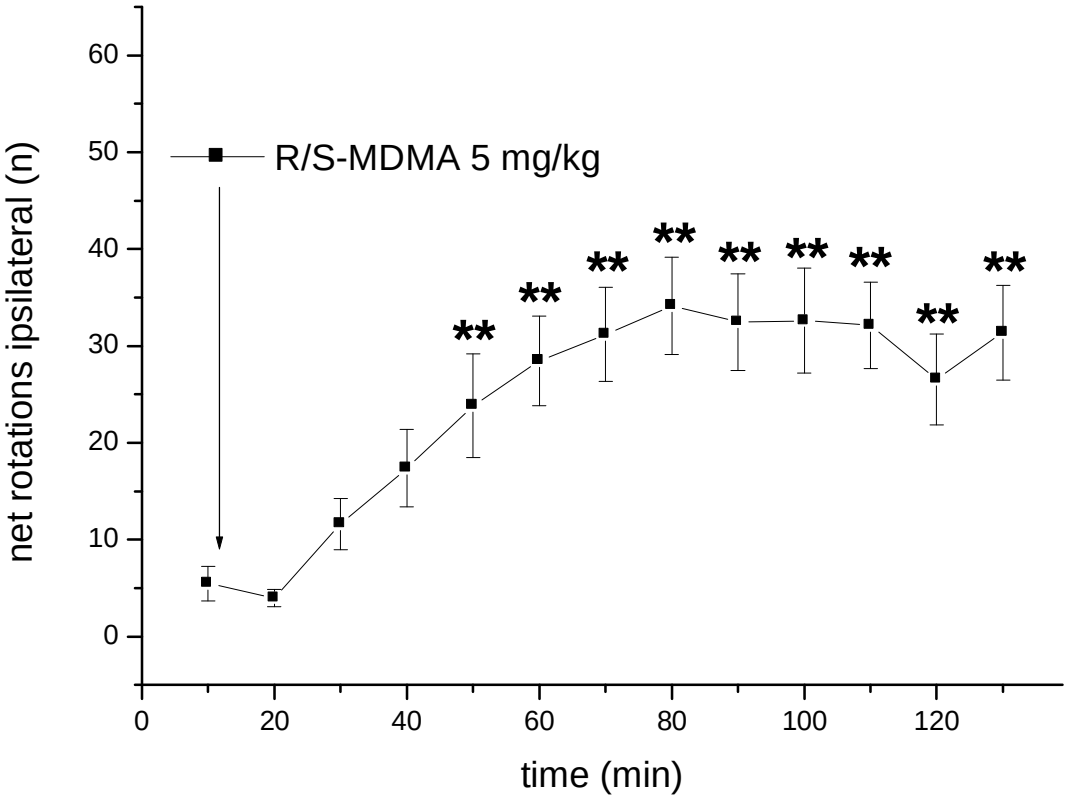

FIG. 4. Time course of rotations of unilaterally 6-OHDA-lesioned rats after treatment with racemic MDMA (n=12). After 10 minutes of baseline recording animals received an injection of MDMA (5 mg/kg). Data are shown as mean ±SEM. **p<0.01 vs. baseline. Net rotations per 10 minutes were analysed by one-way ANOVA with repeated measures followed by post-hoc Tukey/Kramer Procedure t-Test for multiple pair wise comparisons.

Discussion

Dopamine hypofunction and in turn parkinsonian symptoms like akinesia and rigidity were induced by treating rats with the dopamine receptor blocker haloperidol. Haloperidol-induced catalepsy is a reliable animal model for parkinsonism and is selectively reversed by all clinically effective antiparkinsonian drugs. Catalepsy resembling rigidity and akinesia was measured in two established tests as descent latency of the rat from a horizontal bar and a vertical grid. Injection of 2,5 mg /kg MDMA either as racemic, R- or S-MDMA was able to reverse haloperidol-induced catalepsy measured 30 minutes after treatment and also 30 minutes after a second treatment with the respective other enantiomer or racemic MDMA. Racemic MDMA was effective more pronounced than its enantiomers, R-MDMA seemed to be least effective.

In our previous report (Schmidt et al., 2002) R-MDMA was not able to decrease haloperidol-induced catalepsy, but S-MDMA and racemic MDMA decreased catalepsy at the same dosages as used in the current study. However, the degree of catalepsy was higher in the former study and it may have needed a higher dose of R-MDMA to antagonise catalepsy. Therefore, the anti-cataleptic effect of the enantiomers of MDMA, especially of R-MDMA, is not as reproducible as with racemic MDMA and it is concluded that R-MDMA is least effective.

The rotational behaviour test is one of the most powerful tools to screen potential anti-Parkinsonian agents. It bases on the finding that the level of dopamine in the substantia nigra is very low in Parkinsonian patients and in medial forebrain bundle 6-hydroxydopamine lesioned animals. Potential anti-Parkinsonian agents can either stimulate dopamine release from presynaptic terminals or they can activate postsynaptic dopamine receptors. MDMA caused ipsilateral rotations in a dose-dependent and reproducible manner (Lebsanft et al., 2003). In the current experiment, racemic MDMA and S-MDMA were more potent than R-MDMA.
We have reported about anti-parkinsonian actions of MDMA before (Schmidt et al., 2002, Lebsanft et al., 2003) but the reasons for these effects are unknown so far. Serotonin release, which is reported to be the main neurochemical effect of MDMA, can not be the only cause since enhanced serotonin rather enhances catalepsy (Wadenberg, 1996). There has even been a case report on de novo onset of Parkinsonism in humans after treatment with the selective serotonin reuptake inhibitor citalopram (Stadtland et al., 2000). Serotonin in general has been reported to inhibit firing of dopaminergic neurons from substantia nigra (Kelland et al.,1990, Sinton et al., 1988).

The behavioural differences of racemic MDMA and both of its enantiomers may resemble differences of neurochemical effects.
Both enantiomers are nearly equipotent releasers of serotonin, but the S-enantiomer is about 5-fold more potent in enhancing dopamine release than its congener. This could be one reason for its stronger efficacy in rotational behaviour.
Regarding the affinity of MDMA to 5HT1 and 5HT2 receptors, it has been shown, that the R(-) enantiomer displays higher affinity than the S(+) isomer (Lyon et al., 1986; Schmidt, 1987; Battaglia & De Souza, 1989). Involvement of 5HT2-receptors in some of MDMA's effects has been shown in serotonergic neurotoxicity, acute hyperthermia, corticosterone secretion, disruption of sensorimotor gating, locomotor stimulation, reinforcement (Padich et al., 1996; Schmidt & Kehne, 1990; Schmidt et al., 1990; Nash et al., 1988; Kehne et al., 1996; Fantegrossi et al., 2002). However, this receptor has also been discussed to be involved in MDMA induced dopamine release in rats (Gudelsky et al., 1994; Schmidt et al., 1994; Yamamoto et al., 1995). As R(-)-MDMA was least effective in the current experiment, we conclude that this mechanism is not essential for the antiparkinsonian action of MDMA.
Greater efficacy of the S (+) isomer of MDMA has been demonstrated in human studies and serotonin-release where the S (+) isomer was more effective than the R(-)-isomer (Anderson et al., 1978; Nichols et al., 1982; Schmidt et al., 1986; Schmidt, 1986; Cho et al., 1990; Hiramatsu & Cho, 1990).
The stereoselectivity of MDMA at the serotonin transporter with the higher potency of the S(+)-enantiomer has been demonstrated by Rudnick and Wall (1992) and serotonin transporter deficient mice were insensitive to the locomotor stimulating effects of S(+)-MDMA (Bengel et al., 1998). In general, this mechanism is very important as MDMA enters the cell via the serotonin transporter, this could be one reason why S(+)- MDMA is more effective than R(-)-MDMA. Only the S(+)-enantiomer of MDMA inhibited [3H]-dopamine uptake into striatal synaptosomes and was more effective in releasing [3H]-dopamine and inhibiting reuptake of [3H]-dopamine in vitro; regarding norepinephrine and serotonin, the enantiomers were found to be equipotent (Nichols et al., 1982; Johnson et al., 1986; Schmidt et al., 1987; Steele et al., 1987; Kalix et al., 1988; McKenna et al., 1991). Serotonergic neurotoxicity is also more pronounced with the S(+)-enantiomer, this effect has also been discussed to be dopamine-dependent (McKenna et al., 1991). S(+)-MDMA was also more potent at eliciting stereotyped behaviours (Hiramatsu et al., 1989), disrupting operant responding in mice (Rosecrans & Glennon, 1987) and in producing hyperthermia in rats than its R(-) enantiomer. The S(+)-enantiomer was also more potent in mimicking the

discriminative stimulus produced by racemic MDMA (Battaglia et al., 1988; Schechter, 1987). Regarding the discriminative stimulus effects, similar to amphetamine (Schechter, 1978; Young & Glennon, 1986), the S(+)-enantiomer is more potent than the R(-)-enantiomer (Schechter, 1987, Glennon et al., 1988). In addition, racemic MDMA was observed to be more potent than either of the two isomers (Schechter, 1987). Locomotor stimulation produced by MDMA is also mainly attributable to the S(+)-enantiomer (Glennon et al., 1988). There are indications that the discriminable cue of S(+)-MDMA is more dopaminergic whereas the stimulus properties of R(-)-MDMA are more serotonergic (Glennon et al., 1988, Baker & Taylor,1997; Fantegrossi et al., 2002). In all these effects the S(+)-MDMA is more effective as with our current results. But all these behaviours are susceptible to manipulations at various transmitter systems including dopamine or opioids (Hiramatsu et al., 1989).
It is known that systemic administration of S(+)-MDMA but not R(-)-MDMA increases extracellular levels of dopamine (Yamamoto & Spanos, 1988; Hiramatsu & Cho, 1990; Gough et al., 1991; Schmidt et al., 1991), this is an argument for dopaminergic mediated processes as cause for the anti-parkinsonian effects seen in our current experiments. Reported in vivo dopamine release time course (Nash, 1990; Nash & Yamamoto, 1992; Gough et al., 1991; Gudelsky & Nash, 1996) in striatum corresponds with time course of rotation in 6-hydroxydopamine lesioned MDMA treated rats in the current experiment and earlier reports (Lebsanft et al., 2003). A similar time course was reported for serotonin release by Shankaran and Gudelsky (1999). In another report, dopamine and serotonin release is starting earlier than onset of rotation in our experiment and it resembles the time course of d-amphetamine induced dopamine release (Sabol & Seiden, 1998; Gudelsky et al., 1994). This strong dopamine releasing effect is only reported for the S(+)-enantiomer of MDMA and not correlated to formation of MDA from MDMA (Hiramatsu & Cho, 1990, Hiramatsu et al., 1991; Schmidt et al., 1991) though levels of MDA after the S(+)-enantiomer are about 3 times higher (Hiramatsu & Cho, 1990), especially in cortex and striatum (Meyer et al., 2002). MDA has been shown to be more potent in releasing dopamine than MDMA itself. But there is a temporal correlation between MDMA levels and dopamine efflux, but not MDA, indicating that the acute dopamine effects are due to the parent compound with little contribution from the metabolite MDA (Hiramatsu et al., 1991). It has also been shown that the S(+)-enantiomer produces long lasting serotonin neurotoxicity (Schmidt, 1987), a fact that is also known for MDA (Ricaurte et al., 1985). Endogenous dopamine has also been discussed to play a role in the serotonergic deficits produced by administration of MDMA (Stone et al., 1988).
A variety of drugs that influence cholinergic, noradrenergic, GABAergic, opioid and substance-P systems of the brain can induce turning responses, when investigated either following lateralized intracerebral administration or given peripherally to animals with a variety of unilateral central lesions (Pycock, 1993). The direction of rotations after administration of MDMA was the same as after injection of amphetamine which is thought to mediate this effect by releasing dopamine from presynaptic terminals. Predominantly amphetamine-like rotation has also been observed in unlesioned animals after administration of MDMA (Kulmala et al., 1987). Amphetamine shares some behavioural properties with MDMA but the two substances also differ in other behavioural and neurochemical effects and MDMA is therefore thought to represent a class of drugs different from psychomotor stimulants (Nichols et al., 1986). Zetterstrom and colleagues have demonstrated that ipsilateral rotation in response to amphetamine in unilaterally 6-hydroxydopamine denervated rats correlated well with time-course of dopamine release in striatum contralateral to the lesion. MDMA resulted in stimulus generalization to amphetamine (Glennon, 1986).The ratio for serotonin release to dopamine relesase is much greater for MDMA than for amphetamine (Crespi et al., 1997), although MDMA, like amphetamine, does release a larger amount of dopamine (Gudelsky & Nash, 1996; White et al., 1994; Yamamoto & Spanos, 1988). Increasing evidence suggests that amphetamine-enhanced extracellular dopamine level is due

to blockade of vesicular monoamine transporter. This results in an increased intracellular level of dopamine that is released by the synaptic dopamine transporter by reverse transport (Fon et al., 1997, Jaber et al., 1997, Jones et al., 1998, Wang et al., 1997). A similar mechanism of MDMA on serotonin- and dopamine release is probable as it has been shown that MDMA inhibits vesicular uptake of monoamines (Hansen et al., 2002, Bogen et al., 2003, Mlinar & Corradetti, 2003).
Compared to amphetamine, MDMA produces a different pattern of locomotion (Paulus & Geyer, 1991) and suppresses rearing behaviour (Gold et al., 1988; Callaway et al., 1990). Like amphetamine, MDMA has been shown to be a competitive inhibitor of MAO-A activity and a mixed inhibitor of MAO-B with comparable kinetics. But in contrast to amphetamine, the inhibition of MAO-A has not been found to be stereoselective for the S(+) enantiomer with MDMA (Mantle et al., 1976, Kokotos Leonardi & Azmitia, 1994). MAO-A inhibition has been discussed to enhance dopamine release through enhanced serotonin levels by an exchange-diffusion mechanism (Jacocks & Cox, 1992, Kokotos Leonardi & Azmitia, 1994).
In conclusion, MDMA counteracts parkinsonian symptoms. S(+)- and racemic MDMA are more potent dopamine releasers than R(-)-MDMA. Therefore they are most potent in the rotational behavioural model, since this models dopamine-deficiency only. Parkinson´s disease affects not only the dopaminergic system but many other structures as well (Braak et al., 2002). Therefore the anticataleptic (Schmidt et al., 2002) and anti-Parkinsonian (Margolis, 2001) efficacy of MDMA and the R- enantiomer is probably due to effects of non dopaminergic mechanisms as well.

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3,4-Methylenedioxymethamphetamine and naloxone in rat rotational behaviour and open field

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Abstract

It has been shown recently that 3,4-Methylenedioxymethamphetamine (MDMA) has symptomatic antiparkinsonian activity in rodent models of Parkinson´s disease. The mechanism of this anti-parkinsonian action is unknown so far.
Opioids have been suggested to play a role in MDMA induced behaviour.
We therefore investigated MDMA and naloxone in the rat rotational behavioural model. Male Sprague Dawley rats were lesioned unilaterally with 6-hydroxydopamine at the medial forebrain bundle. Administration of R/S-MDMA (5 mg/kg, s.c.) produced ipsilateral rotations. Naloxone (2,5,10 mg/kg, s.c.) did not produce rotations on its own but reduced the number of MDMA-induced ipsilateral rotations. This effect was not dose-dependent. In contrast to reports on mice, in unlesioned animals, naloxone (10 mg/kg, s.c.) did not block MDMA (5 mg/kg, s.c.)-induced hyperactivity in an open field in our experiment.
It is concluded that endogenous opiates play a role in MDMA's action in the rat rotational behavioural model.

Abbreviations: ANOVA analysis of variance, GABA gamma-aminobutyric acid, MDA 3,4-Methylenedioxyamphetamine, MDMA 3,4-Methylenedioxymethamphetamine, MFB medial forebrain bundle, 6-OHDA 6-Hydroxydopamine, PBS: phosphate-buffered saline, SEM standard error of the means.

Introduction

3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is a very popular recreational drug, it has gained popularity over the past 20 years because of its ability to produce strong feelings of euphoria, empathy, and connection to others [51, 17]. It is most frequently used orally and rarely snorted. MDMA is declared illegal in the Schedule I in the International Convention on Psychotropic Substances.
MDMA is chemically related to amphetamine-like stimulants and hallucinogens [45].
Its most prominent acute pharmacological effect is to block the reuptake of serotonin and reverse the flow at the serotonin transporter resulting in enhanced release of serotonin from nerve terminals [41,36]. Additionally, MDMA causes transporter-mediated release of central dopamine and norepinephrine.
In rodents, it elicits dramatic increases in locomotion. As MDMA is a releaser of both serotonin and dopamine [36, 42, 35], it is not clear so far if the locomotor-stimulating effects are due primarily to direct effects on dopamine release or indirectly by the influence of

serotonin release upon dopamine transmission [13, 14, 23]. Interactions between serotonergic and dopmaninergic systems of the brain have been described on numerous investigations. There is evidence that MDMA increases dopamine release through carrier-mediated processes, but also that released serotonin increases dopamine release via stimulation of 5-HT_2 receptors.
However, not all effects of MDMA are explained by these mechanisms of action. The anti-parkinsonian action of MDMA and its derivates is not fully understood yet. We have shown recently, that MDMA induces ipsilateral rotation in 6-hydroxydopamine unilaterally lesioned rats [32] and that MDMA counteracts catalepsy [44]. These effects could not be fully explained by serotonergic or dopaminergic activity of the drug.
It has been shown recently, that enkephalin contributes to the locomotor stimulating effects of MDMA in mice [19]. μ-receptor activation by morphine is known to induce burst-like increases of locomotion [2]. On the other hand, morphine is known to induce catalepsy at higher doses [50, 20].
Here the question is addressed to which extent μ-receptor activation is involved in the locomotor stimulating effects of MDMA in rats in an open field and in the MDMA induced ipsilateral rotation in unilaterally 6-OHDA lesioned rats.

Material and Methods

Animals

All rats (male, Sprague-Dawley, 7-8 weeks old and 226 - 250g body weight at the beginning of the experiments (Charles River, Germany)) were acclimatized and handled by the experimenter for about one week before start of the experiments. The rats were housed in groups of six in a temperature- 22,5°C RT and humidity- 55% controlled environment under a 12/12 hours light-dark cycle. Water was available ad libitum and standard rat food was delivered once daily at 12 g per animal. All experiments were carried out during the light phase and were performed in accordance with international ethical standards and the German Animal-Protection Law and have been approved by the local animal care committee (Tierschutzkommission, Regierungspräsidium Tübingen, ZP 05/01).

Drugs

All drugs were freshly dissolved on the day of testing and administered subcutaneously in an injection volume of 1 ml/kg. Racemic MDMA HCl was kindly supplied by the Pharmaceutical Institute, Department of Pharmaceutical Chemistry/Analysis, University of Tuebingen, Germany and dissolved in phosphate-buffered saline (PBS, Sigma, Taufkirchen, Germany). MDMA HCl was administered at 5.0 mg/kg. Naloxone hydrochloride dihydrate (Sigma, Taufkirchen, Germany) was dissolved in PBS and administered at doses of 2, 5 and 10 mg/kg respectively.

Open field experiment

Rats were treated with PBS vehicle (n=10), MDMA 5 mg/kg (n=10), naloxone 10mg/kg (n=10), or combined MDMA 5 mg/kg and naloxone 10mg/kg (n=10). The respective drugs were injected one hour before behavioural analysis. During that time, animals were kept in their home cages in the experimental room. Animals were placed gently into the plastic boxes of a light-beam rodent activity box monitoring system (47cm x 47cm x 44cm, TSE, Technical & Scientific Equipment GmbH, 61350 Bad Homburg, Germany) and locomotive behaviour and rearings were recorded over a time span of 10 minutes.
The path length travelled, number and time span of rearings and time active and inactive by each animal was recorded.

Rotational experiment

After acclimatization rats were lesioned by 6-OHDA application into the medial forebrain bundle (MFB):
Twentyfour Rats were anesthetized with Pentobarbital-Natrium (Narcoren®, Merial Hallbergmoos, Germany) 60mg/kg, i.p. and 6-OHDA HBr (8µg in 1µl ascorbic acid 0.01%, both Sigma Taufkirchen, Germany), was injected into the left medial forebrain bundle at 0.1µl per min. The stereotaxic coordinates were: A = -4.0 mm from the interaural line, L = 1.6mm from bregma and H = 8.8 mm from the surface of the skull according to a stereotaxic atlas [38]. The injection cannula was left in place for additional 4 minutes to allow diffusion of the neurotoxin. 30min prior to the lesion; desipramine HCl (Sigma Taufkirchen, Germany) was administered i.p. at a dose of 20mg/kg to protect noradrenergic neurons against damage by 6-OHDA. Atropinesulfate (0.2mg per animal in 0.2ml saline, Sigma Taufkirchen, Germany) was administered subcutaneously to ease breathing during the anaesthesia. To allow recovery from surgery, animals were singly housed for one day after the surgery. The retrograde degeneration of the dopaminergic cells originating in the Substantia nigra was allowed to fully develop during the next 29 days. During that period, the rats were kept in their home cages. All compounds were tested in the same rats, in experiments lasting 130min with a wash-out period of at least 5 days between each treatment. For each treatment rats were regrouped in a counterbalanced manner respective to their pre-treatment.
Animals were placed into the plastic bowls of a rotameter system (TSE, Technical & Scientific Equipment GmbH, 61350 Bad Homburg, Germany) and baseline rotational behaviour (360 degree turns) was recorded over a time span of 10 minutes. Immediatelly afterwards, rats were injected subcutaneuosly with MDMA respectively naloxone and rotational bahviour was recorded for further 120 minutes. Rotations in the ipsilateral (counter clockwise) and contralateral (clockwise) directions were counted separately and the analyses were based on the net scores (counter clockwise minus clockwise rotations); a positive score indicated that the animals exhibited a net ipsilateral bias.
Data were recorded by the TSE rotameter software as *.dat file and stored as *txt in ASCII format. Export files *.csv compatible to *.xls (Microsoft EXCEL) were generated.

Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA) followed by post-hoc tests using GB STAT version 7.0.
Data are given as means ± SEM and a p-value of 0.05 was accepted as significant.
For comparison of several treatments in the rotational experiment the results were analysed by two-way ANOVA with repeated measures design followed by Fisher's LSD protected t-Test for multiple pair wise comparisons.
The measurements of distance, running time, number of rearings and rearing time in the open field experiment were analysed using two factor ANOVA for completely randomized groups. Post hoc comparisons were done with Tukey/Kramer Procedure.

Results

The ability of naloxone to affect MDMA (5mg/kg) evoked behaviour was assessed in two rat models.
Ipsilateral rotations in 6-OHDA hemilesioned rats were significantly higher after MDMA treatment ($F_{4,55}$= 6.179, p=0,0004) in the time span from one hour to two hours after application of the respective drugs. This time span was selected for statistical analysis because the rotational peak effect was achieved after one hour.
Naloxone did not induce rotations in 6-OHDA hemilesioned rats but significantly diminished rotations induced by MDMA. As Naloxone in dosages 2 mg, 5 mg and 10 mg per kg all had the same effect, this reduction of MDMA-induced ipsilateral rotations is not dose-dependent. The results of the rotational experiment are depicted in Fig. 1.

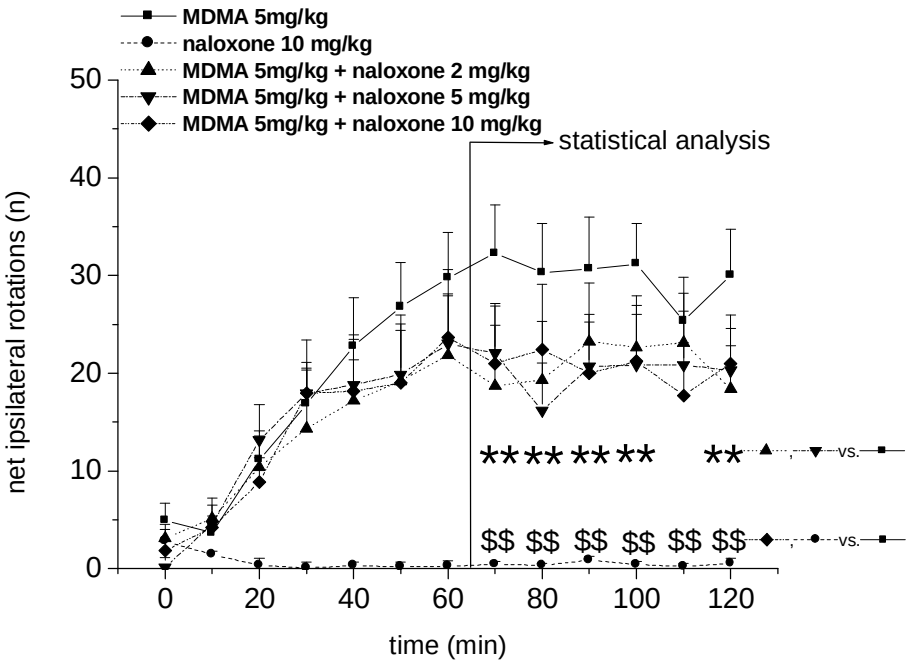

FIG. 1. Naloxone administration (2,5, or 10mg/kg) decreases MDMA (5mg/kg)-induced ipsilateral rotations in unilaterally 6-OHDA MFB lesioned rats (means ± SEM, n= 12), (F(4,55)= 6.179, p=0,0004). Naloxone did not induce rotations on its own. Rats were treated with the respective drugs after 10 minutes of baseline recording. **, $$ indicates p< 0,01 (Fisher's LSD protected t-Test for multiple pair wise comparisons) versus respective group.

In the open field experiment, MDMA stimulated locomotion; it augmented the total distance travelled ($F_{1,36}$= 175.860, p<0,0001) from 35m in vehicle treated rats to 87m in MDMA treated animals. Running time in MDMA treated animals was also significantly augmented ($F_{1,36}$= 318.129, p<0,0001), the number of rearings only showed a tendency to be increased by MDMA. Naloxone treatment did not produce any differences in open field behaviour compared with vehicle treatment. Under combined MDMA and naloxone treatment, naloxone did not change the effects of MDMA: distance travelled and running time were increased compared to vehicle treatment and naloxone treatment but not significantly different from MDMA treatment. The levels of significance of the individual treatments are shown in Fig. 2.

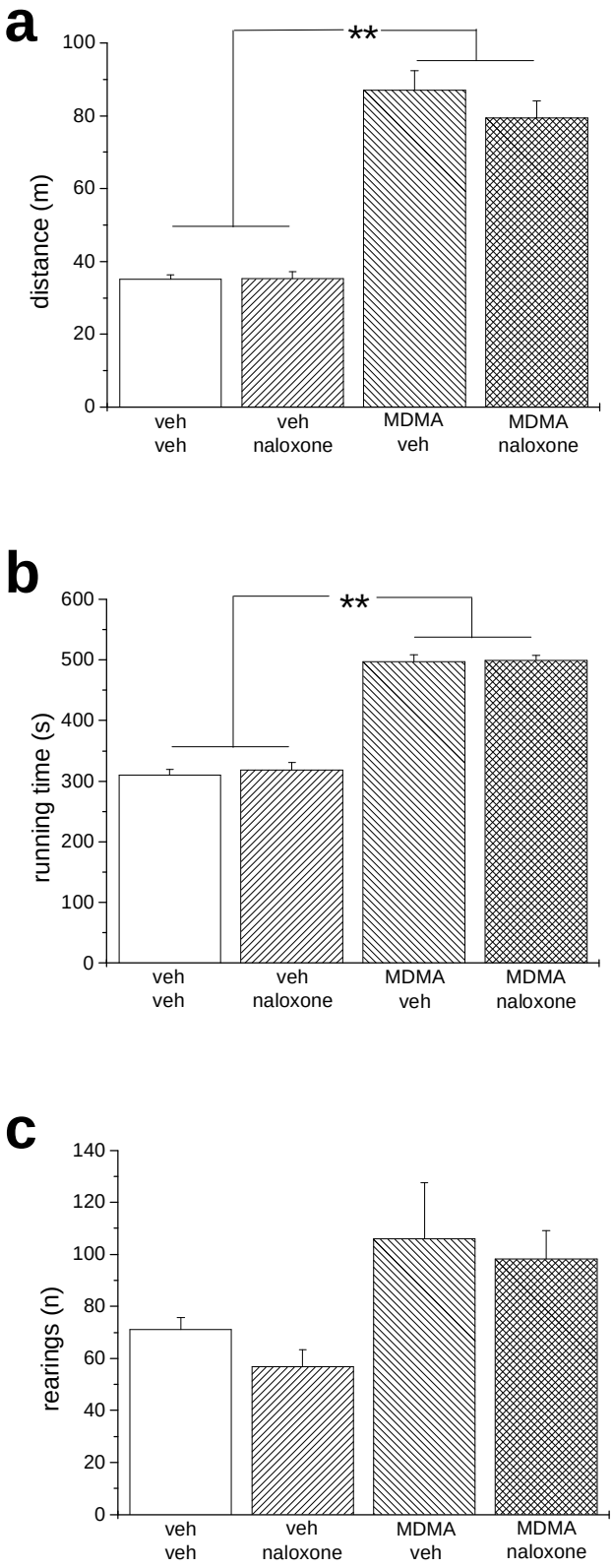

FIG. 2. Naloxone administration (10mg/kg) does not influence MDMA (5mg/kg)-induced hyperlocomotion in rat open field behaviour (means ± SEM, n= 10). Naloxone did not induce hyperlocomotion on its own. a. MDMA treatment augmented the total distance travelled ($F(1,36)= 175.860$, $p<0,0001$) b. Running time in MDMA treated animals was also significantly augmented ($F(1,36)= 318.129$, $p<0,0001$). c. The number of rearings only showed a tendency to be increased by MDMA. . ** indicates $p< 0,01$ post hoc comparisons with Tukey/Kramer Procedure versus respective group.

Discussion

In the current experiments administration of racemic MDMA (5 mg / kg) produced ipsilateral rotations in unilaterally 6-OHDA lesioned rats. This is consistent with our previous reports [32]. Naloxone did not produce rotations on its own but partly reduced the number of MDMA-induced ipsilateral rotations. This effect was not dose-dependent. In contrast to this effect on rotation and to reports on mice [19], naloxone did not block MDMA-induced hyperactivity in an open field in our experiment with unlesioned rats. We found that naloxone treatment itself alone did not induce hyperactivity or rearings in male Sprague-Dawley rats. This in accordance with Kuzmin et al. [30] and Compan et al. [18] who reported no hyperactivity or increased rearing in mice treated with naloxone.
The locomotor stimulating effect of MDMA assessed as increase of distance travelled and running time has been reported several times before. In our experiment the number of rearings only showed a tendency to be increased by MDMA, rearings have been reported to be increased or decreased inconsistently.
Our different results regarding MDMA and naloxone in unlesioned and unilateral dopamine lesioned rats are argumentation that MDMA´s anti-parkinsonian effect may not simply resemble its locomotor stimulating properties.
The locomotor stimulating effects of MDMA in the open field may be due to its amphetamine-like properties, these appear to be mediated mainly by dopamine release.
It is known that systemic administration of MDMA increases extracellular levels of striatal dopamine [48, 49, 54, 25, 33, 34, 35, 30]. In vivo dopamine release time course [34, 35, 25] in striatum corresponds with time course of rotation in 6-OHDA hemilesioned MDMA treated rats in this experiment and earlier reports [32]. This enhancement of release and blockade of metabolism of dopamine and serotonin resembles the mechanism of amphetamine [25]. Zetterström and colleagues [56] have demonstrated that ipsilateral rotation in response to amphetamine in unilaterally 6-OHDA denervated rats correlated well with time-course of dopamine release in striatum contralateral to the lesion. However, the raise in dopamine concentration after MDMA is independent of cell firing since haloperidol did not block this effect [50, 17, 43]. Conversely, dopamine antagonists [28] or dopamine depletion with 6-OHDA [24] attenuated the behavioural effects of MDMA.
It was postulated earlier, that MDMA´s effect on striatal DA function in vivo is partially dependent on endogenous serotonin because when it was blocked by pre-treatment with fluoxetine, the ability of MDMA to increase extracellular dopamine was significantly attenuated [10, 30] and depletion of serotonin with parachlorophenylalanine attenuated the increase of extracellular striatal dopamine produced by MDMA [11] (Brodkin et al. 1993). We have reported that blocking serotonin release by treatment with Citalopram or depletion of serotonin with parachlorophenylalanine also attenuates rotational behaviour in 6-OHDA lesioned rats elicited by MDMA [32] . Additionally, it is known that intrastriatal application of either serotonin or serotonin receptor agonists increases extracellular striatal dopamine [6, 7, 18, 22, 37, 53, 52, 27, 30]. The facilitating effect of serotonin release on dopamine release is dependent on intact serotonergic –and perhaps other transmitter systems since it is not evident in striatal slices [30]. It has also been discussed that MDMA induced increase in the extracellular concentration of dopamine in the striatum is mediated in part by 5-HT_2 receptors as ketanserin inhibits MDMA induced dopamine release in vivo [34]. This effect was only prominent in a higher dose of MDMA, indicating that at lower doses the amphetamine like actions of MDMA increase dopamine release via carrier-mediated mechanisms.
Hyperactivity reported after administration of MDMA has also been suggested to be the result of activation of 5-HT_{1B} receptors [15, 16, 40]. The enhanced dopamine release may result from disinhibition of GABAergic neurons via 5-HT_2- and/or 5-$HT_{1B/1D}$- receptors that are stimulated by endogenous serotonin [55].

Bankson and Cunningham [3] suggested that 5-HT_{2C} receptors mask dopaminergically mediated hyperactivity induced by actions of MDMA via 5-$HT_{1B/1D}$ receptors and therefore blockade of these receptors further potentiates hyperactivity. However, it is possible that direct dopamine release contributes to the hyperlocomotion seen after MDMA administration. MDMA is known to increase serotonin release but also to enhance dopamine efflux [9, 47, 28, 42, 54, 33, 35, 30]. MDMA is a very "dirty" drug acting at many different receptors and transporters, at the postsynaptic side. There may also be a convergence of different neurotransmitter systems at the level of G proteins .

MDMA binds to different brain recognition sites including serotonin reuptake sites, serotonin receptors, histamine receptors, muscarinic receptors, dopamine receptors, NMDA receptors [4, 21] and has been shown to induce acetylcholine release [21, 1]. Furthermore, MDMA has been shown to act as agonist at the rat trace amine receptor [12]. In our experiment, we further investigated the role of endogenous opiates in MDMA induced locomotion and rotational behaviour in rats.

Behaviours are susceptible to manipulations at various transmitter systems including dopamine or endogenous opioids.

Opioids play a role in these behaviours as μ- and δ-opioid antagonists have been shown to reduce dyskinesia in parkinsonian primates [26]. Thus, MDMA induced elevated dopamine level is not only due to direct release but also to an indirect influence by other transmitter systems.

The in vitro pharmacological profile for MDMA by Battaglia and colleagues [4, 5] revealed only low affinities (> 500 μM) at μ-, δ- and κ-opioid receptors but it has been shown that opioids modulate MDMA`s reinforcing properties [39, 8] and it has been shown recently, that enkephalin contributes to the locomotor stimulating effects of MDMA in mice [19]. MDMA combined treatment with the opioid antagonist naloxone failed to induce hyperactivity in wild-type mice. [19]. As we cannot confirm these results in the open field for rats, we suppose that MDMA has rather different effects on the endogenous opioids in normal rats and mice. However, for the stimulating properties of MDMA that induce rotational behaviour in 6-OHDA hemilesioned rats, the modulating effects of opioids seem to be comparable to the effects seen on hyperactivity in mice.

The basal ganglia , which are involved in sensorimotor regulation, include the striatum and its main projection areas: the globus pallidus and the substantia nigra. Axon terminals of striatal effernt neurons contain metenkephalin and GABA in the globus pallidus; substance P, dynorphin and GABA in the SN. A decrease in GABA release by the striatopallidal neurons is related to hyperlocomotion, an increase to catalepsy. μ-and δ-Opioid receptor agonists have been shown to increase extracellular dopamine levels in the nucleus accumbens and striatum [46]. In parallel, numerous studies have shown that selective opioid agonists increase locomotion possibly via the striatopallidal neurons. Therefore it is possible that MDMA exerts its locomotor stimulating effects via the endogenous opiates. However, in our experiment this effect was not evident in an open field in normal rats but only became evident in 6-OHDA hemilesioned rats.

There are further possibilities where endogenous opioids may interfere in combination with GABA on locomotor behaviour. The basal ganglia contain the highest density of 5-HT_{1B} receptors in rodents, which are localized on the striatal efferent neurons. Increases in both GABA and substance P are usually correlated with hyperlocomotion, additionally, hyperlocomotion might be related to increases of metenkephalin.

The effect of MDMA on extracellular GABA levels has been investigated in basal ganglia [55]. The nigral GABA release is mediated by the 5-HT_{1B} and 5-$HT_{2A/2C}$ receptors [55]. MDMA induced serotonin release may inhibit GABA release via activation of 5-HT_{1B} receptors, which in turn could attenuate the negative GABA control on metenkephalin metabolism resulting in increased metenkephalin levels. MDMA administration decreased

metenkephalin levels in the globus pallidus, in wild-type but not in 5-HT_{1B} knockout mice, [19] suggesting that the effects of MDMA on metenkephalin metabolism are mediated via activation of 5-HT_{1B} receptors. These effects could be antagonised by application of the opioid antagonist naloxone resulting in decreased hyperlocomotion. It could be possible that these effects are especially important in animals with disturbed dopaminergic transmission like it is the case in 6-OHDA hemilesioned rats. Opioid receptor-agonism could therefore be involved in MDMA induced anti-parkinsonian effects.

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