

Ecstasy counteracts catalepsy in rats, an anti-parkinsonian effect?

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Received 22 April 2002; received in revised form 19 July 2002; accepted 22 July 2002

Abstract

Parkinson's disease is due to a dopamine deficiency caused by the degeneration of midbrain dopamine neurons. Current therapies are aimed to substitute dopamine or to directly stimulate postsynaptic dopamine receptors. However, not all patients profit from current therapies to the same extent, even serious side effects such as dyskinesias are complicating the therapy. Therefore, there is still a need for better anti-parkinsonian drugs. Here we show that some compounds from the 'Ecstasy'-derivatives exert potent anti-parkinsonian activity. 3,4-Methylenedioxymethamphetamine, 'Ecstasy' dose-dependently and very potently reversed haloperidol-induced parkinsonism in the rat. From the supraadditive effect of the enantiomers it may be concluded that both enantiomers contribute to the antiparkinsonian effects at two different target sites.

Keywords: Parkinson's disease; Catalepsy; Haloperidol; Ecstasy; 3,4-Methylenedioxymethamphetamine; 3,4-Methylenedioxyethylamphetamine; 3,4-Methylenedioxyamphetamine; *N*-Methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine; Enantiomers

3,4-Methylenedioxymethamphetamine (MDMA) ('Ecstasy') and related derivatives, which share a basic amphetamine structure, exert stimulatory effects in animals and in humans [5]. Moreover, it has been suggested that methylenedioxy analogues of amphetamine (i.e. 3,4-Methylenedioxyamphetamine, (MDA); MDMA; 3,4-Methylenedioxyethylamphetamine, (MDE); *N*-Methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine, (MBDB); Fig. 1) represent a novel class of drugs, named entactogens, because of their psychic effects [13]. Surprisingly, preliminary data from animal experiments [17] and episodic observations in humans [9] indicate an uniquely good anti-parkinsonian effect of MDMA. This opens the possibility to find a component in MDMA's mechanism of action that might be developed into an effective and safe anti-parkinsonian drug. Thus, the present study was designed to examine the unexpected effects of MDMA in parkinsonian states and to analyze the underlying mechanisms.

Male Sprague–Dawley rats (Charles-River, Sulzfeld, Germany) weighing 220–300 g were used for the experiments. Rats were group-housed and kept under constant

conditions and under a 12:12 h light cycle (lights on 07:00–13:00). All rats had lived in the colony room for at least 2 weeks before the start of the experiment. Water was available ad libitum, animal standard food was delivered once daily at 12 g/animal. For each test group and the respective control group *N* = 10. All experiments were performed between 09:00 and 17:00 and in compliance with international ethical standards and the German Animal-Protection Law. They have been approved by the local animal care committee (Tierschuttkommission, Regierungspräsidium Tübingen, ZP 5/01).

Dopamine hypofunctioning and in turn parkinsonian symptoms in the rat i.e. akinesia and rigidity (catalepsy), were induced with the dopamine receptor blocking drug haloperidol (Haldol[®], Janssen, Germany) which was diluted with phosphate buffered saline (PBS, Sigma Deisenhofen, Germany) to a concentration of 0.5 mg/ml. Haloperidol-induced catalepsy has been proven to represent a reliable model for parkinsonism and is selectively reversed by all clinically effective antiparkinsonian drugs.

The racemic Ecstasy-derivatives MDA, MDMA, MDE and MBDB were synthesized from Piperonal obtaining the hydrochloride salts, according to methods of Braun and colleagues [3]. The enantiomers of MDMA and MDE were obtained by means of fractionised crystallization as salts of di-O-benzoyl-tartaric acid [2] (for further details

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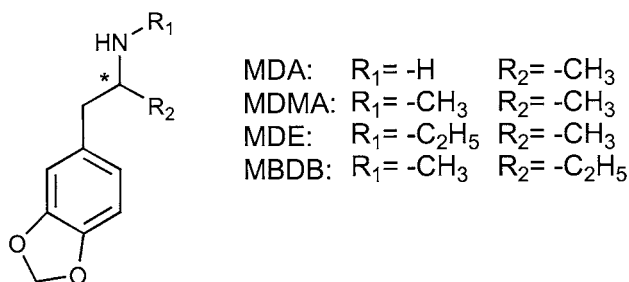


Fig. 1. Structure of MDA, MDMA, MDE and MBDB. Asterisk denotes the chiral centre of the methylenedioxyamphetamines.

see: [4]). The identity of the derivatives and purity of the enantiomers was proved by means of infra red (IR) and mass spectroscopy and ultraviolet-diode-array-detected high pressure liquid chromatography (HPLC-UV-DAD) and assessed as 91% R-MDMA, 97.2% S-MDMA, 99.9% R-MDE and S-MDE, respectively. Finally, the MDMA-derivatives were dissolved in the haloperidol containing PBS to the respective working concentrations. For the catalepsy tests, all rats were injected subcutaneously (s.c.) 60 min before testing with either haloperidol (0.5 mg kg^{-1}) or a combination of haloperidol and one of the methylenedioxyamphetamine-derivative ($1.0, 2.5, 5.0 \text{ mg kg}^{-1}$ R,S-MDMA; 2.5 mg kg^{-1} R-MDMA or S-MDMA resp.; 2.5 mg kg^{-1} R/S-MDA or 5.0 mg kg^{-1} R/S-MBDB; $2.5, 5.0 \text{ mg kg}^{-1}$ R/S-MDE or 5.0 mg kg^{-1} R-MDE or S-MDE resp.).

Catalepsy (rigidity and akinesia) was measured in two established tests in the following order of succession: (1) bar: placing both forepaws on a horizontal bar 9 cm above floor; (2) grid: hanging the animal with all four paws onto a vertical grid. This procedure was repeated two times to avoid side effects like novelty when animals are placed the first time onto the apparatus. The second catalepsy test is represented in the figures. The degree of catalepsy was assessed quantitatively by measuring the time from placement of the animal until removal of one of its paws ('descent latency') with a cut-off time of 180 s. For catalepsy data, non-parametrical statistical tests for two independent groups (Mann–Whitney *U*-tests) were used to detect significant differences between the descent latencies of haloperidol-treated and haloperidol + MDMA-derivative treated groups since the data of catalepsy tests do not follow a normal distribution.

Injection of racemic MDMA-HCl in a dose range from 1 to 5 mg kg^{-1} was able to reverse the haloperidol-induced catalepsy very potently and dose-dependently (Fig. 2). The main neurochemical effects of MDMA are carrier-mediated release of serotonin (5-HT) and norepinephrine (NE) and, with lesser potency, release of dopamine (DA) [15]. From this neurochemical profile however, an anti-parkinsonian effect would not have been predicted since 5-HT release does not counteract parkinsonism: on the contrary, the literature on 5-HT and catalepsy shows that 5-HT release or reuptake inhibition by fluoxetine or clomipramine

enhances catalepsy [21]. In humans, selective serotonin reuptake inhibitors, for example citalopram, can – although rarely – induce de novo onset of parkinsonian symptoms [20]. The procataleptic effects of 5-HT-enhancers are due to a possible inhibition of firing of nigral dopamine neurons [8,18]. Consequently, 5-HT(1A) agonists that reduce firing of 5-HT-neurons by autoreceptor stimulation in the raphe nuclei, do exert anticataleptic activity [12].

All substances of the MDMA-derivatives are equipotent releasers of 5-HT [7], but they differed considerably in their effects on catalepsy: racemic MDE was much less potent (Fig. 3) and MBDB completely failed to show any effects in haloperidol-treated rats (Table 1). In nearly equimolar dosage the substances should produce the same quantities of extracellular 5-HT in the striatum, but only MDMA

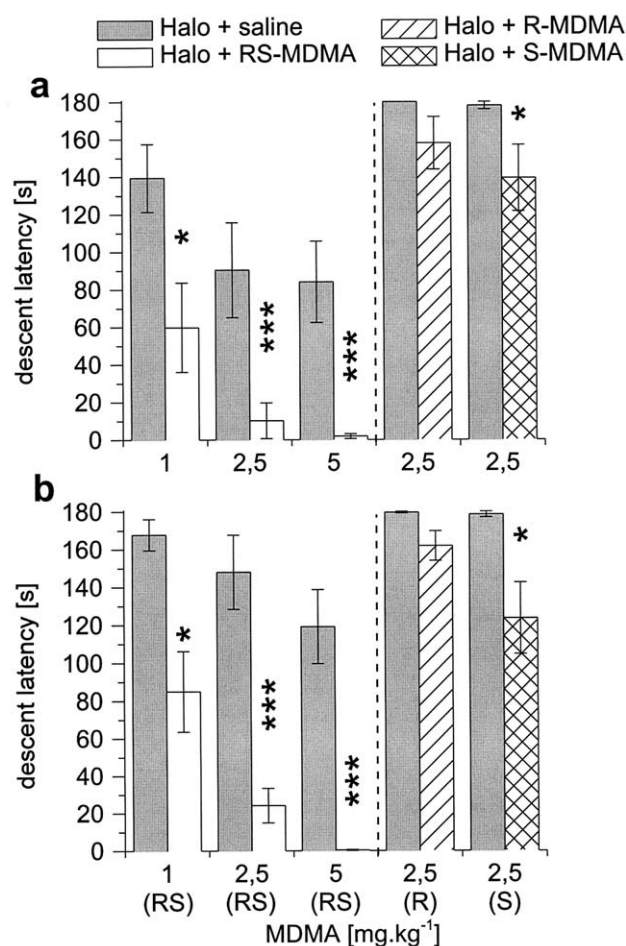


Fig. 2. Anticataleptic activity of MDMA. Descent latencies in seconds (means $\pm$ SEM) of two catalepsy tests ((a): horizontal bar; (b): vertical grid) 60 min after s.c.-injection of 0.5 mg/kg haloperidol + either saline or 1.0 – 5.0 mg kg^{-1} MDMA or 2.5 mg/kg of one of the enantiomers (R-MDMA and S-MDMA). Mann–Whitney *U*-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; (*z*-values: R,S-MDMA: $z_{\text{bar}} = 2.42$, $z_{\text{grid}} = 2.49$ for 1.0 mg kg^{-1} ; $z_{\text{bar}} = 3.36$, $z_{\text{grid}} = 3.40$ for 2.5 mg kg^{-1} ; $z_{\text{bar}} = 3.86$, $z_{\text{grid}} = 3.86$ for 5.0 mg kg^{-1} ; R-MDMA: $z_{\text{bar}} = 1.82$, $z_{\text{grid}} = 2.10$ for 2.5 mg kg^{-1} ; S-MDMA: $z_{\text{bar}} = 2.39$, $z_{\text{grid}} = 2.48$ for 2.5 mg kg^{-1}); $N = 10$ for each group.

Table 1

Descent latencies of animals treated s.c. with either haloperidol (0.5 mg kg^{-1}) with vehicle ('halo + veh') or a combination of haloperidol (0.5 mg kg^{-1}) and either (R,S)-MDA (2.5 mg kg^{-1}) or (R,S)-MBDB (5.0 mg kg^{-1}) ('halo + drug')^a

Drug	Bar		Grid	
	halo + veh	halo + drug	halo + veh	halo + drug
MDA	180.0 ± 0.0	167.0 ± 32.1 (n.s.)	163.0 ± 21.8	127.5 ± 57.8 (n.s.)
MBDB	175.5 ± 14.2	136.7 ± 72.0 (n.s.)	156.4 ± 44.1	121.9 ± 63.5 (n.s.)

^a The animals were tested 60 min after the injection of the drugs in two catalepsy tests (bar and grid). Cut-off time = 180.0 s; $N = 10$ for each group. Means (s) $\pm$ SEM. (n.s.), not significant (Mann-Whitney U -test: $P > 0.05$).

exerted an optimum of anticataleptic action. In summary, it can be assumed that MDMA-induced serotonin release

solely does not account for the anticataleptic effects of MDMA reported here.

The reasonable assumption that direct or indirect actions of MDMA at the nigrostriatal dopaminergic neurotransmission account for the MDMA-induced antiparkinsonian effects can not be completely excluded. However, direct binding of MDMA at D1 or D2 receptors in the striatum can be excluded since MDMA exhibits only very low affinities at dopaminergic receptors [1]. Nevertheless, MDMA also releases DA [10,22]. Nash and Nichols [11] have described previously that the striatal release of DA decreased in the following order of succession: MDA > MDMA > MDE > MBDB. In the present study, treatment with MDA which is the most potent DA-releasing agent among the MDMA-derivatives, did not show any antiparkinsonian effect (Table 1). Thus, it seems rather unlikely that MDMA induced DA release in the striatum accounts for the anticataleptic effects of MDMA.

The finding that MDE which is nearly devoid of any effects on the dopamine system but a strong 5-HT(2) receptor agonist [6,16] was effective in our experiments (Fig. 3), indicates that the 5-HT(2) agonism may contribute to the reversal of catalepsy. Indeed, previous findings of Wadenberg [21] are in accordance with this interpretation: The 5-HT(2)-agonist 2,5-dimethoxy-5-iodoamphetamine, which by itself had no behavioural effects, antagonized raclopride-induced catalepsy. Furthermore, the above mentioned enhancement of striatal DA by MDMA *in vivo* is mediated by 5-HT(2)-receptors, since pre-treatment with the selective 5-HT(2)-antagonist $R\text{-}\alpha\text{-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol}$ (MDL 100,907) markedly attenuated this effect of MDMA [16]. In the present study, the R-enantiomer of MDE, which is a putative 5-HT(2)-receptor agonist [19] was slightly more effective than S-MDE (Fig. 3).

Comparing the effects of the MDMA enantiomers with the racemic drug gives evidence that the mechanism of the anti-parkinsonian actions of MDMA was mediated neither by an indirect DA agonism nor by an agonism at the 5-HT(2)-receptor: racemic MDMA is much more potent than each of the MDMA-derivatives and exerts an even more than additive effect (Fig. 2). Further, S-MDMA showed only weak antiparkinsonian activity while R-MDMA was without any significant effects. The two enan-

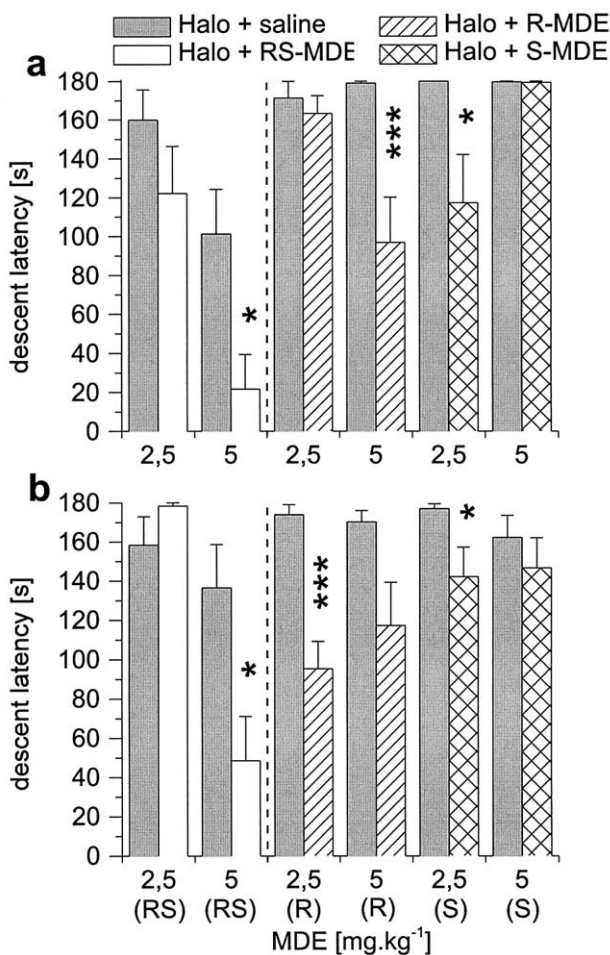


Fig. 3. Anticataleptic activity of MDE. Descent latencies in seconds (means $\pm$ SEM) of two catalepsy tests ((a): horizontal bar; (b): vertical grid) 60 min after s.c.-injection of 0.5 mg/kg haloperidol + either saline or $2.5\text{--}5.0 \text{ mg kg}^{-1}$ racemic R/S-MDE or 5.0 mg/kg one of the enantiomers (R-MDE and S-MDE). Mann-Whitney U -test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; (z-values: R,S-MDE: $z_{\text{bar}} = 0.98$, $z_{\text{grid}} = 0.53$ for 2.5 mg kg^{-1} ; $z_{\text{bar}} = 2.57$, $z_{\text{grid}} = 2.34$ for 5.0 mg kg^{-1} ; R-MDE: $z_{\text{bar}} = 0.92$, $z_{\text{grid}} = 3.67$ for 2.5 mg kg^{-1} ; $z_{\text{bar}} = 3.51$, $z_{\text{grid}} = 1.74$ for 5.0 mg kg^{-1} ; S-MDE: $z_{\text{bar}} = 2.48$, $z_{\text{grid}} = 2.05$ for 2.5 mg kg^{-1} ; $z_{\text{bar}} = 0.07$, $z_{\text{grid}} = 0.71$ for 5.0 mg kg^{-1}); $N = 10$ for each group.

tiomers are nearly equipotent in their effects on the 5-HT release, but differ in their effects on dopamine: The S-enantiomer is about 5-fold more potent in enhancing dopamine concentrations than the R-enantiomer [8]. Only the S-enantiomer of MDE has entactogenic effects in humans [17]. However, the poor effect of the enantiomers on catalepsy indicates that there must be a new unknown component in the MDMA effects which uniquely manifests itself only in the synergism of both enantiomers R- and S-MDMA. Thus, it may be concluded that both enantiomers contribute to the anticataleptic effects at two different target sites.

The use of MDMA-like drugs in the treatment of any disease needs careful consideration: These drugs, like all methamphetamine derivatives, may have an addictive and hallucinogenic potential, further some drugs are neurotoxic. As yet, these dangers can not be fully assessed; but the addictive potential of MDMA may be less pronounced in dopamine-deficiency states, such as Parkinson's Disease. Concerning the serotonergic toxicity, MDE is less toxic than MDMA [11,14] and at the relatively low effective dose against Parkinsonian symptoms is perhaps devoid of toxicity. In conclusion, enantiomers may represent the tools by means of which we hope to discover the anti-parkinsonian component of MDMA.

Supported by Land Baden-Württemberg (Forschungsschwerpunkt Suchtforschung) and Deutsche Forschungsgemeinschaft (SFB 430, Graduiertenkolleg Neurobiologie). The authors wish to thank Mrs Dagmar Waidmann and Mr Uli Ruess for their excellent technical assistance.

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