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Research report

Differential toxic effects of methamphetamine (METH) and methylenedioxymethamphetamine (MDMA) in multidrug-resistant (mdr1a) knockout mice

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Abstract

The toxic effects of methamphetamine (METH) (2.5, 5.0 and 10.0 mg/kg) and methylenedioxymethamphetamine (MDMA) (5.0, 10.0 and 20.0 mg/kg) on dopaminergic systems were assessed in the striatum and of the nucleus accumbens in mdr1a wild-type and knockout mice. METH caused significant dose-dependent decreases of dopamine (DA) and DA transporters (DAT) in the striatum and the nucleus accumbens (NAc) of both wild-type and knockout mice. The lowest doses of METH (2.5 mg/kg) caused only small changes in the wild-type, but marked decreases in the mdr1a knockout mice. The two higher doses (5 mg/kg and 10 mg/kg) caused similar changes in both strains of mice. In contrast to METH, MDMA caused greater percentage decreases in DAT in the wild-type mice. For example, the lowest dose (5 mg/kg) caused significant decreases in DAT in the NAc of wild-type but not of mdr1a knockout mice. The highest dose (20 mg/kg) caused similar changes in both the strains. These results suggest that METH and MDMA interact differentially with P-glycoproteins. These observations document, for the first time, a role for these proteins in the entry of METH and MDMA into the brain via the blood–brain barrier, with P-glycoprotein possibly facilitating the entry of MDMA but interfering with that of METH into the brain.

Keywords: P-glycoprotein; Neurotoxicity; Methamphetamine; Methylenedioxymethamphetamine; Blood–brain barrier; Dopamine; Striatum; Nucleus accumbens; Cancer

1. Introduction

The cellular resistance developed to the toxic effects of drugs with different chemical structures and mechanisms of action is known as multidrug resistance (mdr) [12]. This is related to increased synthesis of phosphoglycoproteins (P-gps). These plasma membrane proteins consist of identical halves each with six putative transmembrane domains (TM) and an intracellular ATP-binding site [5,13]. P-gps are thought to work by extruding a wide range of amphiphilic and hydrophobic drugs from the cell [8,12,13]. The gene families encoding the P-gps have been well characterized both in humans (MDR1 and MDR 3) and mice (mdr1a, mdr1b and mdr2) [7,14,15,19,26,33,34]. The product of mdr1a gene is abundant in the intestine, liver, kidney and the blood–brain barrier (BBB) [7,13,19].

In order to understand the functional involvement of the drug transporting P-gps in the development of drug resistance, homozygous disruption of the mdr1a gene was engineered in mice resulting in mdr1a knockout that have no P-glycoproteins in their BBB [27]. These mdr1a knockouts have been used as models in mechanistic studies seeking to detail the role of P-glycoproteins in the neurotoxicity of certain drugs [27,28]. For example, it has been shown that some lipophilic drugs accumulate several fold higher in the brains of these mice in comparison to wild-type animals [28]. Because of the differential accumulation of these lipophilic drugs in the mdr1a knockout mice, we reasoned that other lipophilic drugs such as metham-

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phetamine (METH) and methylenedioxymethamphetamine (MDMA) might depend on P-glycoproteins to get entry into the brain.

METH is a drug of abuse that causes marked alterations in the dopaminergic systems of rats and mice [1,3,17,21]. It causes decreases in dopamine (DA) uptake sites [25,35], striatal tyrosine hydroxylase activity and neostriatal dopamine levels [18]. MDMA (ecstasy) is a serotonergic neurotoxin in rats [6] but in mice, it is a dopaminergic toxin which does not affect 5-HT systems [4,22,24,32]. Although it has been assumed that these drugs enter the brain avidly because of their lipophilic properties, the role of BBB P-glycoproteins has not been assessed. In the present study, we tested the possibility that METH and MDMA might gain entry into the brain via interaction with P-glycoprotein by assessing whether their toxic effects are affected by the absence of P-glycoproteins in the BBB.

2. Materials and methods

Male mdr1a wild-type (+/+) and knockout (−/−) mice were purchased from Taconic Farms, NY. Wild-type and homozygous mdr1a mice aged 10 weeks were used in these experiments. The animals were given food and water ad libitum. All animal procedures were in accordance with NIH guidelines for the care and use of laboratory animals and were approved by the local NIDA Animal Care Committee.

2.1. Drug treatment with METH and MDMA

2.1.1. Experiment 1

On the day of the experiments, the animals were given intraperitoneal injections of either METH (2.5, 5.0 and 10.0 mg/kg) or saline. METH was given at an interval of 2 h for a total of four injections as described previously [16,31]. One week later, saline and METH-treated animals were sacrificed. One side of the brain was rapidly removed, frozen in isopentane on dry ice and stored at −70°C. Sections (20 μm thick) were cut at −20°C and thaw-mounted on gelatin coated slides. The slides were kept at −70°C until used in autoradiographic assays of DAT labeled with [^{125}I]RTI-121. The striatum from the other side was processed for measurement of dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) by high-performance liquid chromatography (HPLC).

2.1.2. Experiment 2

Mice were treated with MDMA (5, 10, 20 mg/kg) according to the same schedule described above for METH. One week later, the animals were sacrificed and the brain tissues processed as described above.

2.2. HPLC assays

Concentration of striatal DA was quantified by a modified method of HPLC using a Bondapak C-18 column (Waters) combined with electrochemical detection (Bioanalystic Systems (BAS), W. Lafayette, IN) according to established methods [36]. Briefly, the striatum was weighed and diluted with 1 ml of 1% perchloric acid containing 10 ng/ml of internal standard 3,4-dihydroxybenzamine (DHBA). Brain tissue was then disrupted by ultrasonication and centrifuged. Aliquots (15 μl) were injected onto the HPLC system using a Waters WISP 712 automated injection system for the separation of the neurotransmitters.

2.3. Autoradiographic assays

Binding assays were performed essentially as described previously [4]. Briefly, slide-mounted sections were incubated for 60 min at room temperature (RT) with [^{125}I]RTI-121 (80 000 cpm/ml) using a binding buffer consisting of 137.0 mM NaCl, 2.7 mM KCl, 10.41 mM Na_2HPO_4, 1.76 mM KH_2PO_4 and 10 μM NaI. Specific binding was determined in the presence of 10 μM GBR-12909 and represented greater than 90% of total binding. At the end of the incubation period, the slides were washed twice in fresh buffer for 20 min at RT, dipped in ice-cold distilled water and dried under a stream of cool air. The slides were then apposed to radiosensitive films (Hyperfilm, Amersham) with plastic standard (^{125}I, microscales, Amersham) for 3 days at 4°C. [^{125}I]RTI-121 binding in the striatum

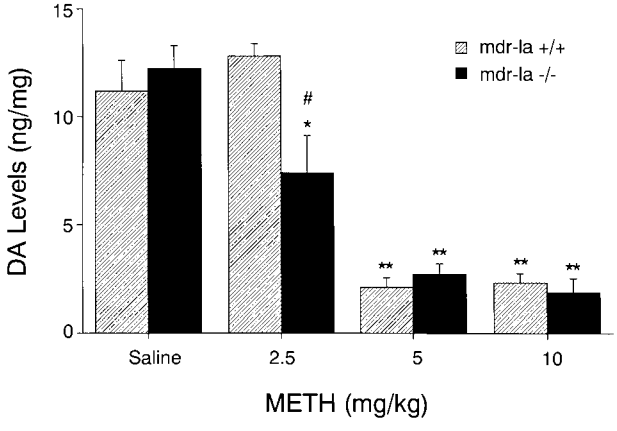

Fig. 1. Toxic effects of METH on striatal DA levels in mdr1a knockout mice. Mice were treated with saline and METH (2.5, 5 and 10 mg/kg × 4). The values represent means ± S.E.M. (ng/mg tissue) of 5–6 animals per group. # $P < 0.0001$ vs. wild-type mice. * $P < 0.001$, * * $P < 0.0001$ vs. saline of same strain.

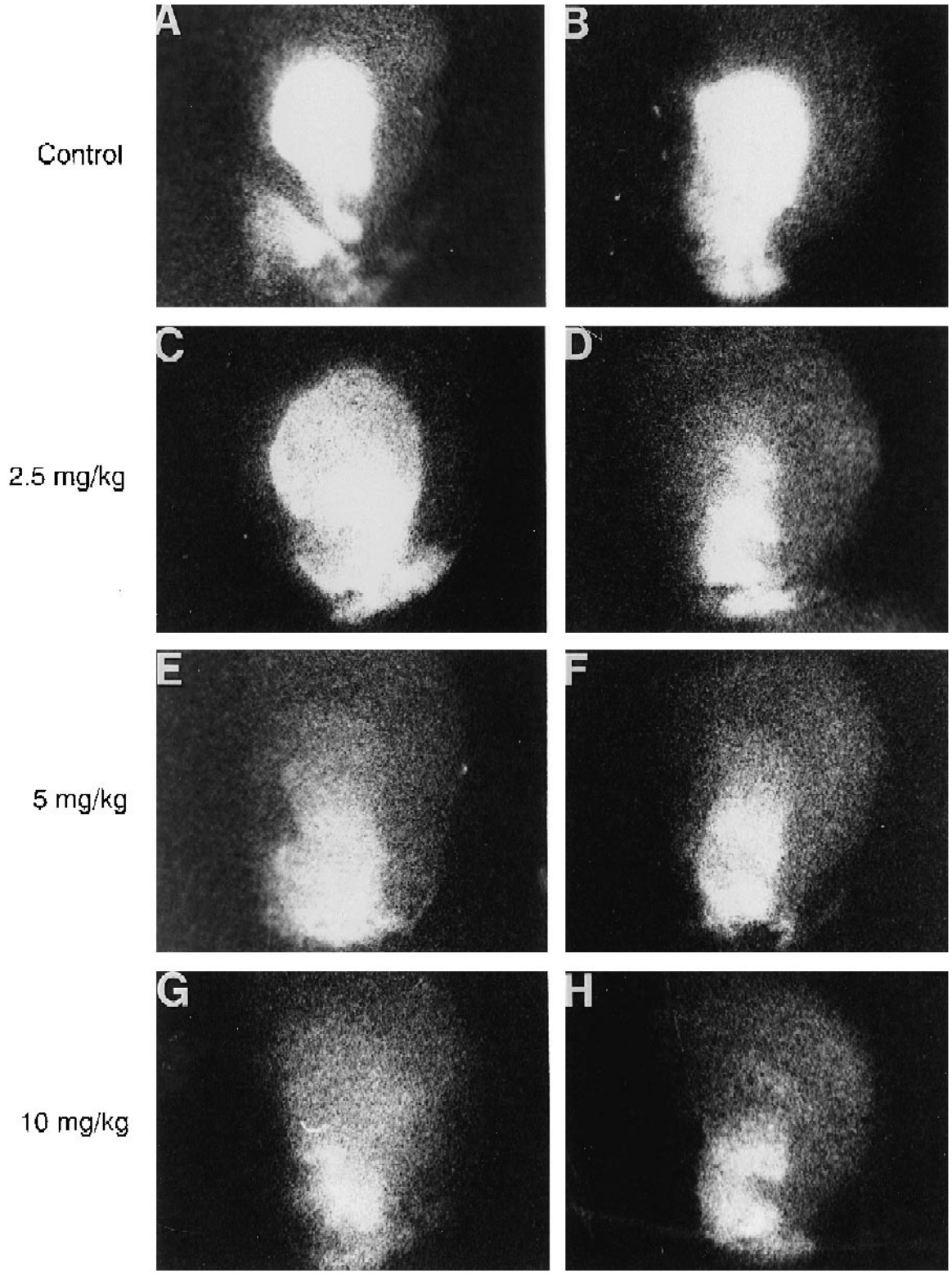

Fig. 2. Autoradiographic representation of [^{125}I]RTI-121 labeled DA uptake sites in striatum of mdr1a +/+ (A, C, E, G) and mdr1a −/− (B, D, F, H). Mice were treated with saline (A, B), METH (2.5 mg/kg × 4) (C, D), METH (5 mg/kg × 4) (E, F) and METH (10 mg/kg × 4) (G, H). Note the obvious decreases in DA uptake site caused by METH at 2.5 mg/kg (C, D) in the mdr1a knockout mice. The quantitative data are given in Fig. 3.

and nucleus accumbens was quantified using a Macintosh computer-based image analysis system (Image, NIH) by using standard curves generated from the ^{125}I-microscales.

2.4. Statistical analyses

The data were analyzed by analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) using Statview 4.02 on a Macintosh (Quadra 840 AV) computer. Criteria for significance were set at the 0.05 level.

3. Results

3.1. Effects of METH on striatal DA levels

METH caused significant dose-dependent decreases in striatal DA levels (Fig. 1). Differences between the two strains of mice were observed only at the lower dose (2.5 mg/kg) of METH. The two higher doses of METH caused similar decreases in DA levels in the two strains of mice.

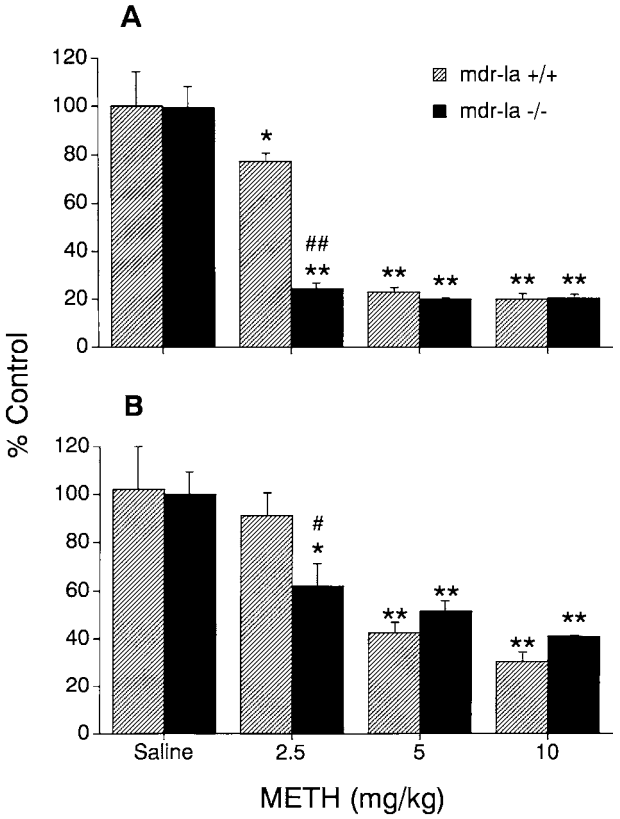

Fig. 3. Toxic effects of METH on striatal DA uptake sites on the striatum (A) and nucleus accumbens (B) of male mice. Mice were treated with saline and METH (2.5, 5 and 10 mg/kg×4). Autoradiographic studies were carried out as described in Section 2. The values represent means ± S.E.M. (% change) of 5–6 animals per group. $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.0001$ vs. wild-type mice. * $P < 0.01$, ** $P < 0.0001$ vs. saline of same strain.

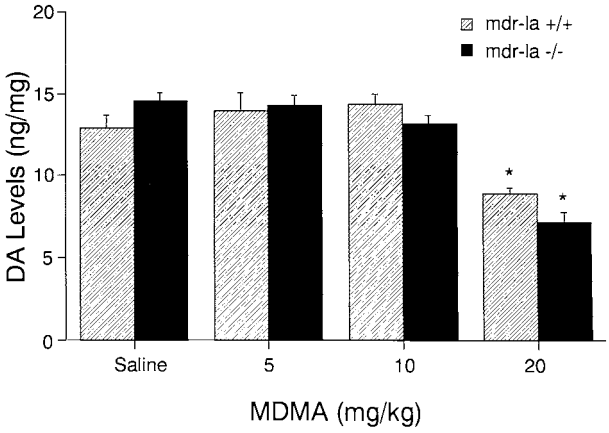

Fig. 4. Toxic effects of MDMA on striatal DA levels of male mice. Mice were treated with saline and MDMA (5, 10 and 20 mg/kg×4). The values represent means ± S.E.M. (ng/mg tissue) of 5–6 animals per group. * $P < 0.001$ vs. saline of same strain.

3.2. Effects of METH on DA uptake sites

The effects of 2.5, 5 and 10 mg/kg of METH on [^{125}I]RTI-121-labeled DA uptake sites are shown in Fig. 2 and Fig. 3. The lowest dose of 2.5 mg/kg METH caused small decreases in DA uptake sites in the striatum (−22.6%) and NAc (−10%) of mdr1a +/+ mice. However, there were marked decreases in the DA uptake sites in the striatum (−76.3%) and NAc (−38.3%) of the mdr1a knockout mice. In contrast, the two higher doses of METH (5.0 and 10.0 mg/kg) caused similar decreases in DA uptake sites in the brains of mdr1a +/+ and mdr1a −/− mice.

3.3. Effects of MDMA on striatal DA levels

MDMA also causes decreases in striatal DA levels (Fig. 4). These decreases were observed only at the highest doses. There were no differences in the effects of the drug on striatal DA levels in the two strains of mice.

3.4. Effects of MDMA on DA uptake sites

MDMA also caused significant decreases in the DAT in the striatum and NAc of mice (Fig. 5 and Fig. 6). However, significant differences were observed between the wild-type and the knockout mice. Specifically, the lowest dose of MDMA (5 mg/kg) did not cause any significant changes in the striatum of either strain of mice, but caused significant decreases (−17.36%) in the NAc of wild-type mice. The middle dose of MDMA (10 mg/kg) caused significant decreases in the striatum (−31.9%) and in the NAc (−37.6%) of wild-type; these decreases were smaller

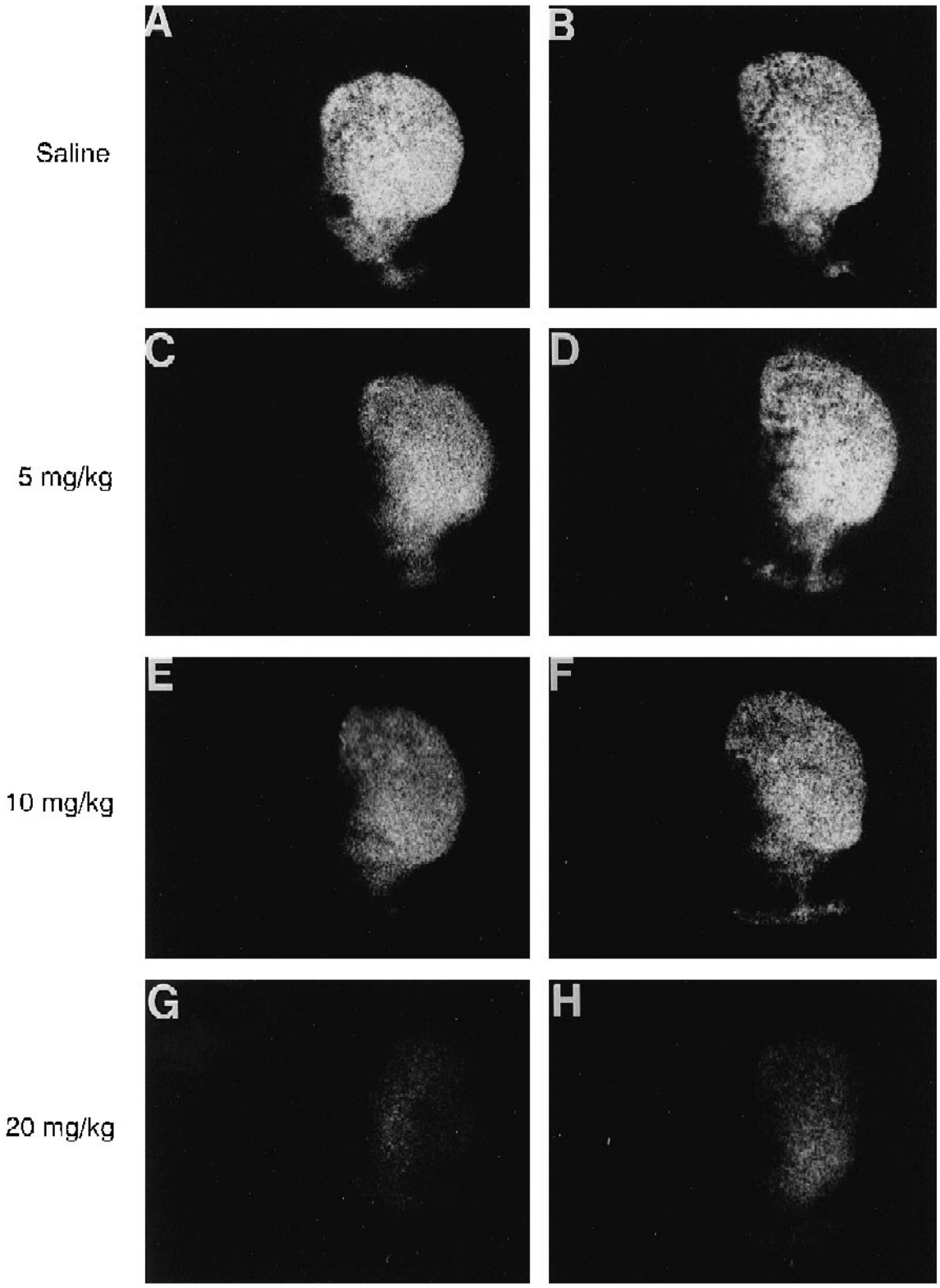

Fig. 5. Autoradiographic representation of [^{125}I]RTI-121 labeled DA uptake sites in striatum of male mdr1a +/+ (A, C, E, G) and mdr1a −/− (B, D, F, H). Mice were treated with saline (A, B), MDMA (5 mg/kg × 4) (C, D), MDMA (10 mg/kg × 4) (E, F) and MDMA (20 mg/kg × 4) (G, H). The quantitative data are given in Fig. 6.

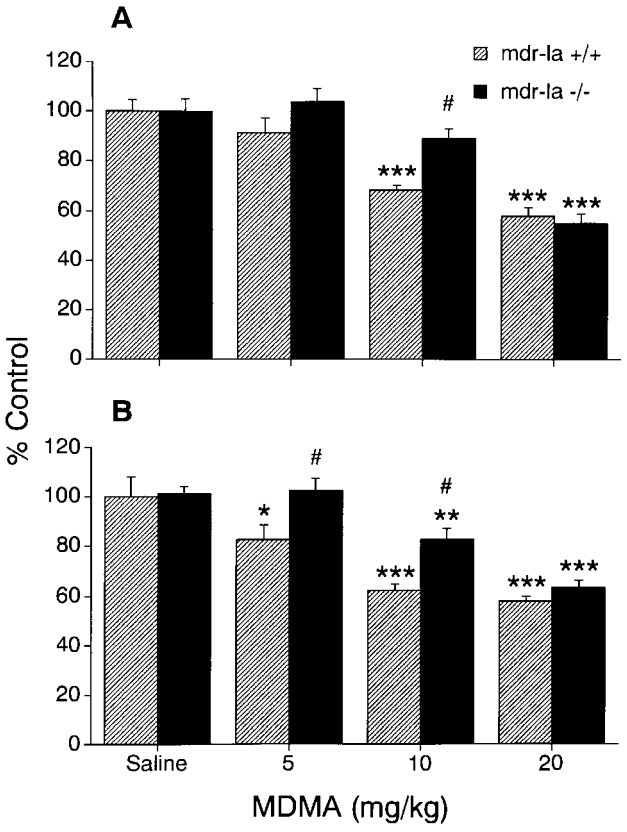

Fig. 6. Toxic effects of MDMA on striatal DA uptake sites on the striatum (A) and the nucleus accumbens (B) of male mice. Mice were treated with saline and MDMA (5, 10 and 20 mg/kg×4). Autoradiographic studies were carried out as described in Section 2. The values represent means ± S.E.M. (% change) of 5–6 animals per group. $^{\#}P < 0.01$ vs. wild-type mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$ vs. saline of same strain.

in the mdr1a knockout mice (Fig. 6). The highest dose of MDMA (20 mg/kg) caused similar changes in both wild-type mdr1a knockout mice.

4. Discussion

The BBB is characterized by the presence of endothelial cells which form tight junctions that prevent the entry of many substances into the brain [11]. Nevertheless, hydrophobic compounds such as ethanol, caffeine and nicotine can easily cross the BBB [2,9,10,20,23,29,30]. It has, thus, been suggested that most lipophilic drugs might gain entry into the brain via passive diffusion. Because of their lipophilicity, drugs of abuse such as the stimulants have been thought to enter the brain via such a mechanism. However, the results of the present study that show differential profile of METH-induced neurotoxicity in the two strains of mice suggest that P-glycoproteins might be involved in controlling the amount of METH that gets into the brain. Thus, in the absence of P-glycoprotein, the smallest dose of METH is enough to cause marked depletion of DA levels and of striatal DAT in the mdr1a knockout. However, high concentrations of METH were able to eliminate this dependency on P-glycoproteins, since both strains of mice were similarly affected when higher doses of METH were used.

In the case of MDMA, the wild-type mice appear to be more sensitive to the toxic effects of the drug. These observations suggest that the absence of P-glycoprotein renders the animals relatively more resistant to the toxic effects of the drugs. These results further suggest that the presence of P-glycoprotein in the BBB might facilitate entry of MDMA into the brain. These observations might be of significant therapeutic values because they suggest that side chain substitutions in anticancer drugs might help to expand the armamentarium against drug-resistant neoplasms.

In summary, this is the first demonstration that mdr1a knockout mice are differentially affected by METH and MDMA. These observations indicate that, in addition to their degree of lipophilicity, interaction of drugs with P-glycoprotein needs to be taken into consideration when their possible entry into the central nervous system is being evaluated.

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