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Differential effect of dietary selenium on the long-term neurotoxicity induced by MDMA in mice and rats

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Abstract

We examined the effect of dietary selenium (Se) on the long-term effect of 3,4-methylenedioxymethamphetamine (MDMA) on dopamine (DA) and 5-hydroxytryptamine (5-HT) containing neurons in the brain of mice and rats. Animals were fed either a Se-deficient (<0.02 ppm) or Se-replete (0.2 ppm) diet for 8 weeks. On the seventh week mice received three injections of MDMA (15 mg/kg, i.p. 3 h apart) or saline and rats a single dose of MDMA (12.5 mg/kg i.p.) or saline. All animals were sacrificed 7 days later. MDMA administration to mice depleted striatal DA concentration in both dietary groups, although depletion was considerably larger in the Se-deficient mice (64%) than Se-replete mice (30%). In addition, a decrease in 5-HT (17–32%) occurred in brain regions of Se-deficient but not Se-replete mice. In rats, MDMA decreased cortical [^{3}H]-paroxetine binding (62%) and 5-HT content, the depletion being similar in the Se-deficient and Se-replete groups. No DA loss occurred in either group. There was no difference in the hyperthermic response induced by MDMA in Se-deficient or Se-replete animals. The Se-deficient diet decreased glutathione peroxidase (GPx) activity by 30% in mouse striatum and cortex and increased the degree of lipid peroxidation in cortical synaptosomes. Se-deficient rats also showed a decrease in brain GPx activity compared with the Se-replete group, but the degree of lipid peroxidation in synaptosomes was similar in both dietary groups. These results suggest that the antioxidant capacity of rats and mice differ leading to a differential susceptibility to the oxidative stress caused by MDMA in situations of low dietary Se.

Keywords: Selenium; Ecstasy; Glutathione peroxidase; Dopamine; Serotonin; Neurotoxicity

1. Introduction

Administration of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') to mice produces relatively selective long-term neurotoxic damage to nigro-striatal dopaminergic pathways, having little effect on 5-hydroxytryptamine (5-HT) containing neurons (Stone et al., 1987; Logan et al., 1988; O'Callaghan and Miller, 1994; Colado et al., 2001; O'Shea et al., 2001). This neurotoxicity has been demonstrated biochemically, there being a sustained loss in the concentration of dopamine (DA) and its metabolites in the striatum. The MDMA-induced loss in the striatal catechol content in mice presumably reflects a neurotoxic degeneration of DA nerve terminals similar to that seen following methamphetamine administration in both rats and mice (Koda and Gibb, 1973; Sonsalla et al., 1989; Green et al., 1992; Baldwin et al., 1993; Bowyer et al., 1994; Itzhak and Ali, 1996).

There have been relatively few studies evaluating the mechanisms involved in the neurotoxic action of MDMA on dopaminergic neurons in the striatum. Nevertheless, all the current evidence indicates that neurotoxicity in mice is due to oxidative stress since: (1) neuronal nitric oxide (NO) inhibitors prevent both neuronal damage and the rise in striatal hydroxyl radical formation induced by MDMA (Colado et al., 2001); (2) CuZn superoxide dismutase (SOD) transgenic mice are resistant to the neurotoxic action of MDMA (Cadet et al., 1995) and; (3) MDMA causes a decrease in SOD and glutathione peroxidase (GPx) activities and an increase in lipid peroxidation in several regions of the brain (Jayanthi et al., 1999). More directly we have recently shown, using in vivo microdialysis, that the

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conversion of salicylic acid to 2,3-dihydroxybenzoic acid (2,3-DHBA) in the dialysate from probes implanted in the mouse striatum is enhanced when animals have been injected with MDMA (Colado et al., 2001; Camarero et al., 2002a). This measure is an accepted method for demonstrating free radical formation (Chiueh et al., 1992; Giovanni et al., 1995; Colado et al., 1997a) and indicates that MDMA is increasing free radical formation in this brain region (Halliwell and Kaur, 1997).

In contrast to its effects in mice, MDMA is generally accepted to behave as a selective 5-HT neurotoxin in rats, inducing a long-term loss of 5-HT nerve terminals in several regions of the brain. The degeneration is reflected by a substantial decrease in the concentration of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), a reduction in the density of 5-HT uptake sites labeled with [^{3}H]-paroxetine (Sharkey et al., 1991; Hewitt and Green, 1994; Colado et al., 1997a; Sanchez et al., 2001) and a decrease in the immunoreactivity of fine 5-HT axons in neocortex, striatum and hippocampus (O'Hearn et al., 1988).

The mechanisms involved in producing this neurodegeneration are not totally understood at present, but recent evidence indicates that a process of oxidative stress is initiated immediately after MDMA administration. By means of intracerebral microdialysis in vivo, it has again been demonstrated that MDMA induces an increase in the formation of hydroxyl radicals, reflected by a rise in 2,3-DHBA in the hippocampal dialysate (Colado et al., 1997a; Shankaran et al., 1999). The hydroxyl radical scavenger α-phenyl-*N*-tert-butyl nitrone (PBN) abolishes the MDMA-induced rise in 2,3-DHBA (Colado et al., 1997a) and attenuates damage to 5-HT neurons (Colado and Green, 1995; Colado et al., 1997a; Yeh, 1999). Additional evidence supporting the existence of an oxidative stress process includes the observation that MDMA increases lipid peroxidation in the brain (Colado et al., 1997b; Sprague and Nichols, 1995).

Selenium (Se) and other antioxidants such as ascorbic acid have been shown to protect nigro-striatal neurons from damage induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) and methamphetamine (Perry et al., 1985; Wagner et al., 1986; Sutphin and Buckman, 1991; Kim et al., 1999; Imam and Ali, 2000; Kim et al., 2000). Se is a micronutrient present in high proportion in some foods of the mammalian diet such as broccoli and forms a vital part of GPx and various Se-dependent enzymes. Specifically, GPx contains a selenocysteine residue in its active site, which is essential for peroxidase activity. Therefore, GPx activity is highly sensitive to dietary Se content to the extent that there is a high correlation between GPx activity, dietary Se levels and resistance to oxidative stress (Castano et al., 1993). GPx together with catalase and SOD are the main cellular antioxidant systems against free radical formation. SOD catalyzes the dismutation of the superoxide anion to hydrogen peroxide (H_2O_2) (Freeman and Crapo, 1982; Fridovich, 1989) while both GPx and catalase use glutathione in the reduction of H_2O_2 to H_2O.

We have now examined the influence of dietary Se on the long-term effect of MDMA on DA and 5-HT containing neurons in the brain of both mice and rats. Animals were fed either a Se-deficient or Se-replete diet for 8 weeks and the GPx activity and degree of lipid peroxidation in the brain were determined. To study the effect of Se supplementation or deficiency on DA and 5-HT pathways, mice and rats received MDMA on the seventh week of dietary treatment. In addition, we evaluated whether the free radical scavenging capability is different in dopaminergic and 5-HT nerve endings in the brain of naive animals. A preliminary account of some of the results was presented at a meeting of the British Pharmacological Society (Camarero et al., 2002b).

2. Methods

2.1. Animals, drug administration and experimental protocol

Thirty-six adult male C57BL/6J mice and 34 adult male Dark Agouti rats (Harlan Iberica, Barcelona, Spain) initially weighing 20–25 and 125–140 g, respectively, were randomly assigned to two dietary treatment groups for 7 weeks. Group I was fed the Se-deficient Torula yeast-based diet (henceforth referred to as Se-deficient diet ($<$0.02 ppm)) while group II received this diet supplemented with 0.2 ppm Se (henceforth referred to as Se-replete diet). The composition of the Se-deficient diet (TD 96163, Harlan Iberica, SL) was as follows (in g/kg): Torula Yeast 300, DL-methionine 3, sucrose 591, corn oil 50, mineral mixture (Se-deficient) 33, calcium carbonate 11 and vitamin mixture Tekland 10. In the case of the Se-replete diet, the above was supplemented with 1 g/kg of a solution of 0.0445% sodium selenite in sucrose (0.2 ppm Se, TD 01116, Harlan Iberica, SL). The Se doses were selected on the basis of earlier studies involving methamphetamine and MPTP (Kim et al., 1999; Kim et al., 2000) and those showing that higher dietary concentrations of Se (1, 2 or 4 ppm) do not suppose a further increase in brain GPx activity (Whanger and Butler, 1988). Mice and rats fed standard maintenance diet were also included. This diet was composed of protein 15.4%, oil 3.5%, fiber 4.1%, ash 5.3% and a mixture of vitamins and minerals containing selenium 0.16 ppm. Mice were housed in groups of three per cage and rats in groups of two, in conditions of constant temperature (21 ± 2 °C) and a 12 h light/dark cycle (lights on 7:00 h) and given free access to their assigned food type and water. Animals were weighed twice weekly and food consumption monitored daily throughout the experiment.

After 7 weeks, groups maintained on Se-deficient (group I) or Se-replete (group II) diet were each divided into two groups ($a + b$). Groups Ia and IIa were administered MDMA: for mice, 15 mg/kg i.p., three times, 3 h apart and for rats a single dose of 12.5 mg/kg i.p., while groups Ib and IIb received saline following the same protocol. MDMA was dissolved in 0.9% w/v NaCl (saline) and injected in a volume of 10 ml/kg for mice and 1 ml/kg for rats. These doses were selected as they have previously been shown to produce a similar degree of depletion in rat cerebral 5-HT (O'Shea et al., 1998, 2002) and mouse striatal DA (O'Shea et al., 2001; Colado et al., 2001; Camarero et al., 2002a). Doses are always quoted in terms of the base. Animals were sacrificed 7 days after MDMA administration. All experimental procedures were performed in accordance with the NIH-Guidelines for the Care and Use of Laboratory Animals. (±) MDMA hydrochloride was obtained from NIDA (Research Triangle Park, North Carolina, USA).

2.2. Measurement of rectal temperature

Immediately before and up to 8 h after the first MDMA injection in mice and up to 6 h after the single dose in rats, temperature was measured by use of a digital readout thermocouple (Type K thermometer, Portec, UK) with a resolution of ±0.1 °C and accuracy of ±0.2 °C attached to a CAC-005 Rodent Sensor which was inserted 2 or 2.5 cm into the rectum of the mouse or rat, respectively, the animal being lightly restrained by holding in the hand. A steady readout was obtained within 10 s of probe insertion.

2.3. Measurement of monoamines and their metabolites in cerebral tissue

Seven days after MDMA administration, animals were killed by cervical dislocation and decapitation, the brains rapidly removed and cortex, hippocampus and striatum dissected out on ice. Tissue was homogenized and 5-HT, 5-HIAA, DA, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) measured by high performance liquid chromatography (HPLC). Catechol concentration was only determined in the striatum since in this area the majority of monoaminergic terminals are dopaminergic. Briefly, the mobile phase consisted of KH_2PO_4 (0.05 M), octanesulfonic acid (0.16 mM), EDTA (0.1 mM) and methanol (16%), and was adjusted to pH 3 with phosphoric acid, filtered and degassed. The flow rate was 1 ml/min and the working electrode potential was set at +0.8 V.

The HPLC system consisted of a pump (Waters 510) linked to an automatic sample injector (Loop 200 μl, Waters 712 WISP), a stainless steel reversed-phase column (Spherisorb ODS2, 5 μm, 150 × 4.6 mm) with a precolumn and an amperometric detector (Waters M460). The current produced was monitored by using an integrator (Waters M745).

2.4. [^{3}H]-paroxetine binding in tissue homogenates

[^{3}H]-paroxetine binding was measured by the method described in detail by Hewitt and Green (1994). The animals were killed, the brain rapidly removed and dissected on ice within 2 min. Cortex from individual animals was homogenized in ice-cold Tris–HCl (50 mM; pH 7.4) containing NaCl (120 mM) and KCl (5 mM) using an Ultra-Turrax. The homogenate was centrifuged at 30,000*g* for 10 min at 4 °C. The supernatant was discarded and the wash procedure repeated twice more. The pellet was finally resuspended in the Tris buffer at a concentration of 10 mg tissue/ml. In order to obtain an estimate of the maximal density of [^{3}H]-paroxetine-labeled 5-HT uptake sites the assay solution (1 ml) contained a saturating concentration of [^{3}H]-paroxetine (1 nM) and 800 μl tissue preparation with the addition of 5-HT (100 μM) for determination of non-specific binding. Incubation was for 60 min at room temperature. Assays were terminated by rapid filtration and counting of the radioactivity by scintillation spectrometry. Protein concentrations were measured by the method of Lowry et al. (1951).

2.5. $FeCl_2$ + ascorbic acid induced lipid peroxidation in vitro

Mouse and rat cortical synaptosomes (P2 fraction) were prepared as described by Lynch and Voss (1990) and resuspended in Tris buffer 50 mM (pH 7.4) containing 120 mM NaCl and 5 mM KCl to produce a protein concentration of approximately 400 μg/ml. Membranes (400 μl) were added to 50 μl $FeCl_2$ (30 μM), 50 μl of ascorbic acid (1 mM) and incubated at 37 °C for 30 min (Colado et al., 1999; Camarero et al., 2002a). Lipid peroxidation was measured by a modification of the method of Das and Ratty (1987), whereby the thiobarbituric acid reacting substances (TBARS), predominantly malondialdehyde, produced as secondary products were quantified by use of the 2-thiobarbituric acid color reaction. Briefly, 150 μl HCl (0.5 M) and 300 μl trichloroacetic acid (40% v/v) were added to the incubation followed by 300 μl 2-thiobarbituric acid (2% w/v). Samples were heated for 15 min at 90 °C and centrifuged at 1,500*g* at 4 °C for 15 min. The pink color developed by the reaction was measured by recording the absorbance at 532 nm and the malondialdehyde concentration calculated by the use of a standard curve prepared with malondialdehyde tetrabutylammonium salt. Assays were performed in triplicate.

2.6. GPx activity

Cortical, striatal and hippocampal tissues were homogenized in 10 volumes of ice-cold sucrose (0.32 M) and centrifuged at 100,000*g* for 30 min at 4 °C. An aliquot of the supernatant was used immediately to measure Se-dependent GPx activity according to the procedure described by Flohe and Gunzler (1984). The assay mixture contained 40 mM potassium phosphate buffer (pH 7), 40 μM EDTA, 1 mM GSH, 0.24 U/ml glutathione reductase, 1 mM sodium azide and supernatant (80 μl). The mixture was preincubated for 10 min at 37 °C. Next, 0.15 mM NADPH was added and the hydrogen peroxide-independent consumption of NADPH monitored for 5 min. The overall reaction was started by the addition of 0.25 mM H_2O_2 and the decrease in absorption at 340 nm monitored for 5 min in a thermostated spectrophotometer (37 °C). The enzymatic activity was calculated from the linear slopes of decreasing absorption as micromole NADPH oxidized per minute using the appropriate extinction coefficient. A blank in which the supernatant was replaced by potassium phosphate buffer was subtracted from each assay. Protein was measured by the method of Lowry et al. (1951).

2.7. Statistics

Data from the monoamine, binding, GPx activity and lipid peroxidation studies were analyzed using one-way ANOVA followed by the Newman–Keuls multiple comparison test when a significant *F*-value was obtained. Statistical analyses of the temperature measurements were performed using the statistical computer package BMDP/386 Dynamic (BMDP Statistical Solutions, Cork, Eire). Data were analyzed by ANOVA with repeated measures (program 2V) or, where missing values occurred, an unbalanced repeated measure model (program 5V) was used. Both used treatment as the between subjects factor and time as the repeated measure. ANOVA was performed on both pre- and post-treatment data.

3. Results

3.1. Mouse studies

3.1.1. Effect of MDMA on weight gain and food consumption of mice fed either a Se-deficient or a Se-replete diet

The initial mouse body weight was similar in both treatment groups (21.0 ± 0.5 g, $n = 18$ and 21.4 ± 0.4 g, $n = 18$ for Se-replete and Se-deficient groups, respectively). There was no difference in the diet consumption between mice maintained on either a Se-deficient or a Se-replete diet during the 7 weeks before MDMA administration (Fig. 1(a)). Both groups consumed an average of 2.9 ± 0.05 and 2.9 ± 0.05 g/day of food, respectively. Nevertheless, the Se-deficient group showed a smaller weight gain than the Se-replete group throughout the dietary treatment period (Fig. 1(b)).

MDMA was administered on the seventh week of dietary treatment, mice being maintained with Se-deficient or replete diet for a further week. Repeated MDMA administration (15 mg/kg) three times at 3 h interval only produced a decrease in diet consumption in the 24 h following injection, the effect being similar in both the Se-deficient and Se-replete groups. This effect was not evident in mice injected with saline (Fig. 1(c)). During the 7 days after saline administration mice maintained on an Se-deficient diet continued to show a reduced weight gain compared with those maintained on an Se-replete diet. MDMA induced a decrease in body weight in both groups of animals which was greater in Se-deficient mice (Fig. 1(d)).

3.1.2. Effect of MDMA on rectal temperature of mice fed with a Se-deficient or a Se-replete diet

Repeated administration of MDMA (15 mg/kg) three times at 3 h interval produced a hyperthermic response lasting over 8 h which was similar in Se-deficient and Se-replete groups. In the Se-replete group the increase was modest after the first dose but enhanced and sustained following the second and third injections of MDMA (Fig. 2). In the case of the Se-deficient group there was an increase only after the second and third injection of MDMA (Fig. 2), resulting in similar total areas under the curve to those in the Se-replete group (Fig. 2, inset). There was no significant difference between the rectal temperature of mice maintained on either Se-deficient or Se-replete diet and given saline (Fig. 2).

3.1.3. Effect of MDMA on the concentration of DA in the striatum and 5-HT in striatum, cortex and hippocampus from mice fed either a Se-deficient or a Se-replete diet

Repeated administration of MDMA (15 mg/kg) three times at 3 h intervals produced 7 days later, a loss in the striatal concentration of DA and its metabolites, DOPAC and HVA in all three groups of animals, but this effect was largest in Se-deficient mice (Fig. 3(a) and (b)). While MDMA administration failed to alter the 5-HT concentration in the hippocampus, cortex and striatum in either standard diet mice or Se-replete mice, it produced a significant long-term loss in the indole concentration in these regions when given to Se-deficient mice (Table 1).

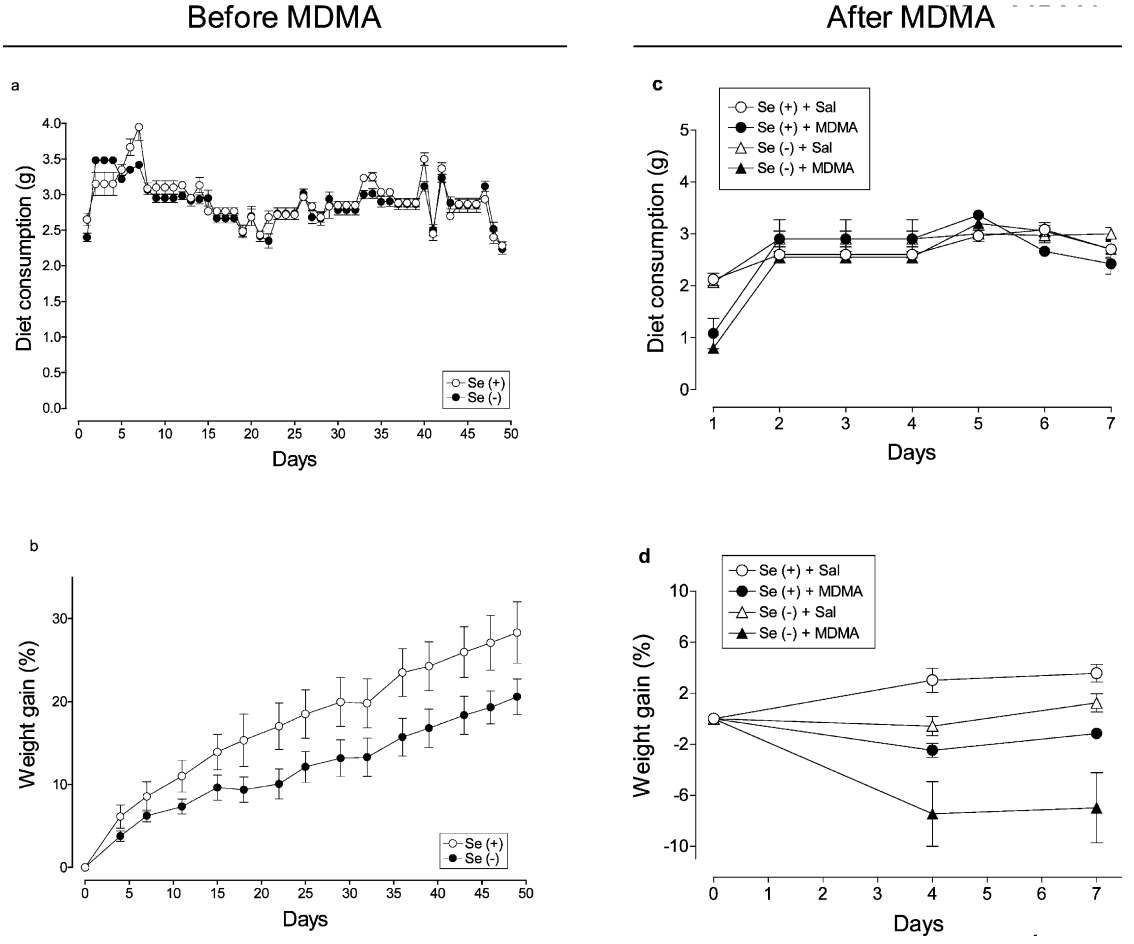

Fig. 1. Diet consumption (a,c) and increase in body weight (b,d) of mice fed either a Se-replete (Se+) or Se-deficient (Se−) diet. Animals were maintained on dietary treatment for 7 weeks (a,b). Then, each dietary group was in turn divided into two groups, which received MDMA (15 mg/kg, i.p.) three times at 3 h intervals or saline, respectively. Animals were maintained on dietary treatment for a further week before sacrificing (c,d). Results are shown as mean ± SEM (n = 12–18 before MDMA and n = 5–9 after MDMA administration). (b) Se (−) diet produced a decrease in the weight gain compared with the Se (+) group [$F(1,26) = 4.9$, $P < 0.05$)]. (c) MDMA decreased the diet consumed by both Se (+) and Se (−) groups compared with saline at 24 h ($P < 0.001$ for both diet groups). (d) MDMA also produced a decrease in weight gain in both the Se (+) [$F(1,8) = 46.7$, $P < 0.001$] and Se (−) [$F(1,9) = 20.2$, $P < 0.001$] groups.

3.1.4. GPx activity and lipid peroxidation in the brain from mice fed either a Se-deficient or a Se-replete diet

GPx activity was reduced by 30% in the cortex and striatum of mice maintained during 8 weeks on a Se-deficient diet (Fig. 4(a)). In addition, 8 weeks after starting treatment the degree of lipid peroxidation, reflected as malondialdehyde formation, was higher in the cortical synaptosomes of mice maintained on low Se compared with the Se-replete group (Fig. 4(b)).

3.1.5. GPx activity and lipid peroxidation in the brain from mice fed a standard diet

In order to study whether the radical trapping capability is different in dopaminergic and 5-HT nerve endings, we determined GPx activity and lipid peroxidation in the striatum (where the majority of monoaminergic terminals are dopaminergic) and the cortex of mice fed a standard diet. GPx activity had a tendency to be lower in the cortex (Fig. 5(a)) and malondialdehyde formation was higher in striatal than in cortical synaptosomes (Fig. 5(b)).

3.2. Rat studies

3.2.1. Effect of MDMA on weight gain and food consumption of rats fed either a Se-deficient or a Se-replete diet

The initial rat body weight was similar in both treatment groups (110.3 ± 3.7 g, n = 16 and 106.4 ± 3.1 g, n = 18 for Se-replete and Se-deficient groups, respectively). There was no difference in the diet consumption between rats maintained with either a Se-deficient or a Se-replete diet during the 7 weeks before MDMA administration (Fig. 6(a); 12.0 ± 0.12 and 11.7 ± 0.11 g/day, respectively). In addition, both groups showed the same weight gain throughout the treatment (Fig. 6(b)).

MDMA (12.5 mg/kg) administration in the seventh

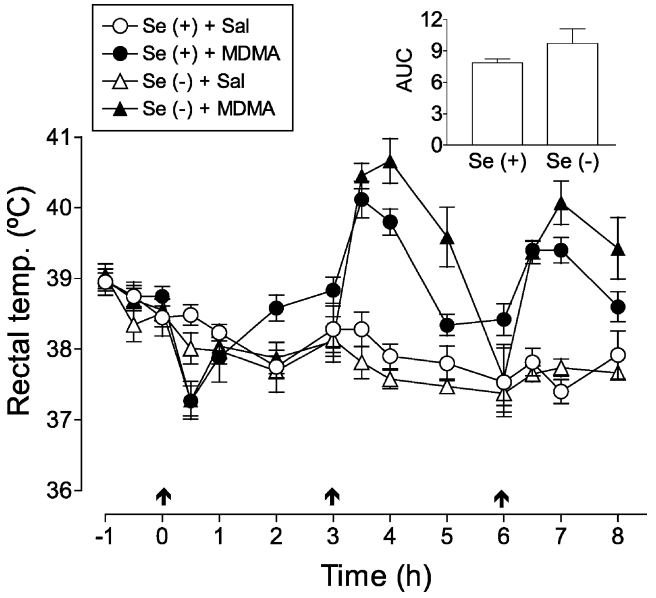

Fig. 2. Rectal temperature of mice fed either a Se-replete (Se+) or Se-deficient (Se−) diet and given three injections of MDMA (15 mg/kg, i.p.) at 3 h intervals. Animals were maintained on dietary treatment for 7 weeks before receiving MDMA and for 1 week after. MDMA produced an increase in the temperature of both Se (+) [$F(1,10) = 26.2$, $P < 0.001$] and Se (−) [$F(1,16) = 70.2$, $P < 0.001$] mice, the hyperthermic response being similar in both groups [$F(1,13) = 1.35$, n.s.]. Results are shown as mean ± SEM (n = 6–9).

week produced a decrease in food consumption only in the first 24 h following injection, the effect being similar in both the Se-deficient and Se-replete groups. This effect was not observed in rats injected with saline (Fig. 6(c)). During the 7 days after MDMA administration, rats in both diet groups showed a reduced weight gain compared with their corresponding saline-treated groups (Fig. 6(d)).

3.2.2. Effect of MDMA on rectal temperature of rats fed a Se-deficient or a Se-replete diet

Administration of MDMA (12.5 mg/kg) produced a hyperthermic response lasting over 6 h which was similar in Se-deficient and Se-replete groups, reaching a peak of 1.8–2.0 °C between 30 and 60 min after administration (data not shown). There was no significant difference between the rectal temperature of rats maintained with either Se-deficient or Se-replete diet and given saline (data not shown).

3.2.3. Effect of MDMA on the concentration of DA and 5-HT and the binding of [^{3}H]-paroxetine in the brain tissue from rats fed either a Se-deficient or a Se-replete diet

Administration of MDMA (12.5 mg/kg) produced 7 days later, a loss in the cortical, hippocampal and striatal concentrations of 5-HT and 5-HIAA. This loss was similar in all three dietary groups (Table 2). MDMA also produced a reduction in the binding of [^{3}H]-paroxetine

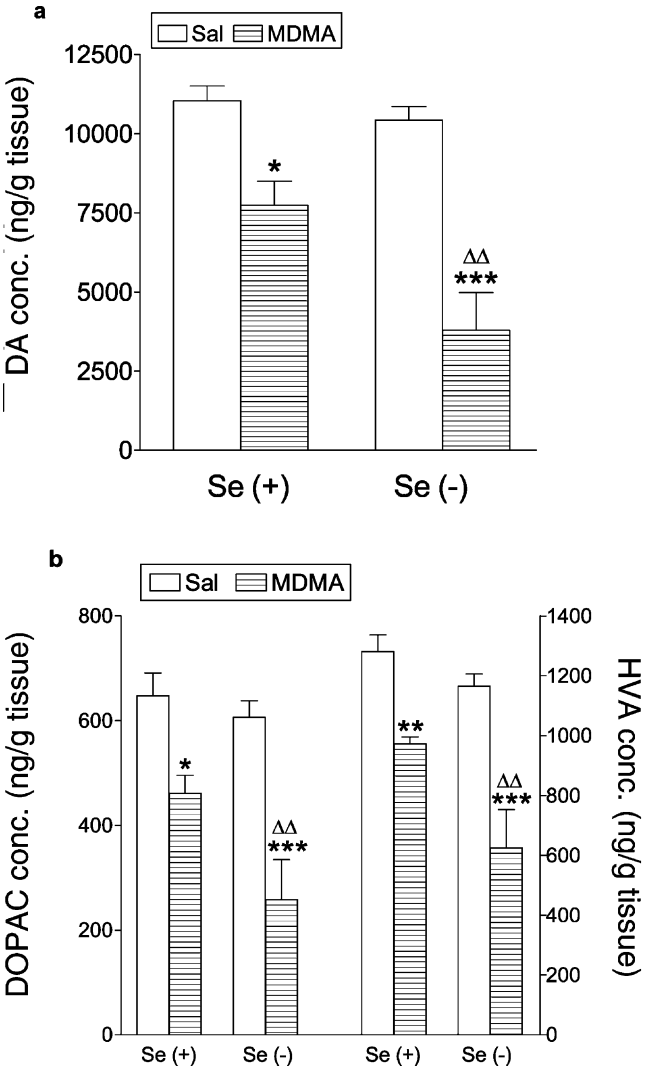

Fig. 3. Concentrations of (a) DA and (b) DOPAC and HVA in the striatum of mice fed either a Se-replete (Se+) or Se-deficient (Se−) diet and given three injections of MDMA (15 mg/kg, i.p.) at 3 h intervals. Animals were maintained on dietary treatment for 7 weeks before receiving MDMA and for 1 week after. Results are shown as mean ± SEM (n = 5–9). Different from the corresponding saline-group: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Different from Se-replete + MDMA group: $^{\Delta\Delta}P < 0.01$. Striatal catechol concentration (ng/g tissue) in mice fed a standard diet was as follows: DA (saline = 10,398 ± 339, MDMA = 6638 ± 538), DOPAC (saline = 680 ± 68, MDMA = 559 ± 53) and HVA (saline = 928 ± 30, MDMA = 774 ± 41).

to 5-HT uptake sites which was the same in all groups (Table 2).

Treatment with MDMA had no effect on the concentration of DA and its metabolites, DOPAC and HVA in the striatum in either dietary group (Table 3).

3.2.4. GPx activity and lipid peroxidation in the brain from rats fed either a Se-deficient or a Se-replete diet

GPx activity was reduced by 35–45% in the cortex, hippocampus and striatum of rats maintained during 8 weeks on an Se-deficient diet (Fig. 7(a)). Eight

Table 1
Concentrations of 5-HT and 5-HIAA in cortex, hippocampus and striatum of mice fed either a Se-replete (Se+) or Se-deficient (Se−) diet and given three injections of MDMA (15 mg/kg, i.p.) at 3 h interval

	Diet	Saline	MDMA
Cortex			
5-HT (ng/g tissue)	Se (+)	315 ± 24	279 ± 23 (11↓)
	Se (−)	287 ± 21	196 ± 8∗,∗∗∗ (32↓)
	Standard	344 ± 11	285 ± 22 (17↓)
5-HIAA (ng/g tissue)	Se (+)	169 ± 10	151 ± 7 (11↓)
	Se (−)	164 ± 11	111 ± 6∗∗,∗∗∗ (32↓)
	Standard	131 ± 6	128 ± 10 (2↓)
Hippocampus			
5-HT (ng/g tissue)	Se (+)	503 ± 10	518 ± 29 (1↑)
	Se (−)	526 ± 26	425 ± 19∗,∗∗∗ (29↓)
	Standard	476 ± 27	441 ± 11 (7↓)
5-HIAA (ng/g tissue)	Se (+)	393 ± 24	402 ± 23 (1↑)
	Se (−)	427 ± 37	301 ± 18 (30↓)
	Standard	340 ± 11	300 ± 14 (12↓)
Striatum			
5-HT (ng/g tissue)	Se (+)	475 ± 31	444 ± 25 (7↓)
	Se (−)	450 ± 8	372 ± 13∗,∗∗∗ (17↓)
	Standard	338 ± 31	319 ± 33 (6↓)
5-HIAA (ng/g tissue)	Se (+)	352 ± 30	318 ± 25 (10↓)
	Se (−)	344 ± 21	245 ± 6∗,∗∗∗ (29↓)
	Standard	245 ± 26	223 ± 21 (9↓)

Animals were maintained on dietary treatment for 7 weeks before receiving MDMA and for one further week. The figure in parenthesis is the percentage of change vs. the respective control group. Results are shown as mean ± SEM (n = 5–9). Different from the corresponding saline-group: $^{*}P < 0.05$, $^{**}P < 0.01$. Different from Se-replete + MDMA group: $^{***}P < 0.05$.

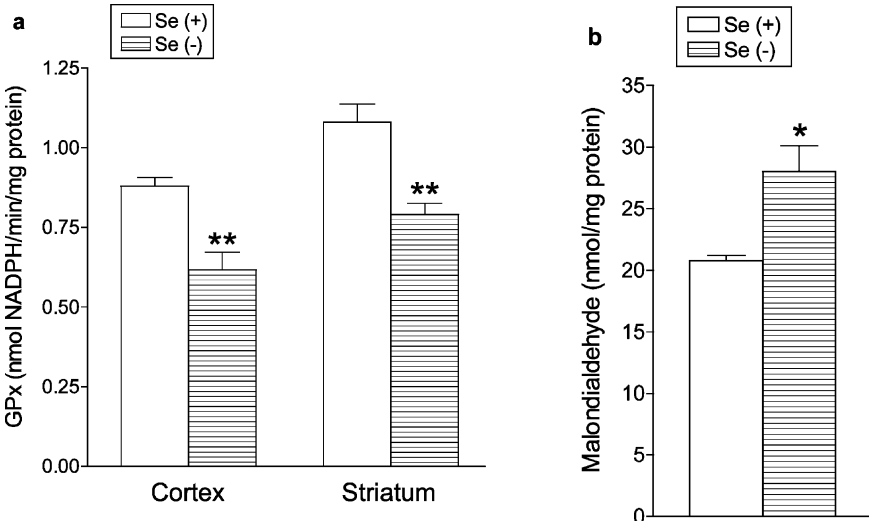

Fig. 4. (a) GPx activity in cortex and striatum and (b) lipid peroxidation in cortical synaptosomes of mice fed either a Se-replete (Se+) or Se-deficient (Se−) diet for 8 weeks. Malondialdehyde formation in vitro was induced by incubation of synaptosomes with $FeCl_2$ (3 μM) + ascorbic acid (0.1 mM). Results are shown as mean ± SEM (n = 4–7). Different from Se-replete + saline group: $^{*}P < 0.05$, $^{**}P < 0.01$.

weeks after starting diet treatment there was no difference in the degree of lipid peroxidation in the cortical synaptosomes of rats in either dietary group (Fig. 7(b)).

3.2.5. GPx activity and lipid peroxidation in the brain from rats fed a standard diet

In order to study whether the radical trapping capability is different in dopaminergic and 5-HT nerve

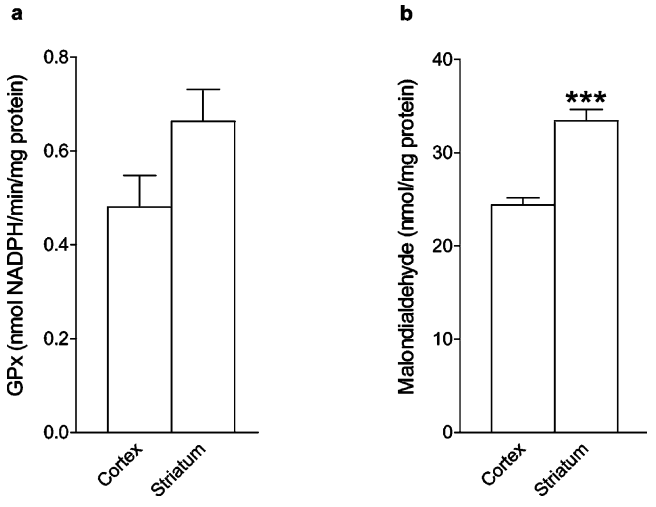

Fig. 5. GPx activity (a) and (b) lipid peroxidation in striatum and cortex of mice fed a standard diet. Results are shown as mean ± SEM (n = 7–8). Different from striatum: $^{***}P < 0.001$.

endings, we determined GPx activity and lipid peroxidation in the cortex, hippocampus and striatum of rats fed a standard diet. There was no difference in either GPx activity (Table 4) or malondialdehyde formation (Table 4) in the three brain areas examined.

4. Discussion

These results indicate that a variation in the content of dietary Se produces qualitative and quantitative changes in the neurotoxicity of MDMA in mice. Thus, mice fed an Se-deficient diet (<0.02 ppm) show an enhancement of the long-term decrease in DA content compared with mice fed the Se-replete diet (0.2 ppm). Our results also show that a dietary treatment practically devoid of Se decreases GPx activity in the mouse brain (by 30% in cortex and striatum), this reduction being enough to induce an increase in the degree of lipid peroxidation in cortical synaptosomes as reflected by an

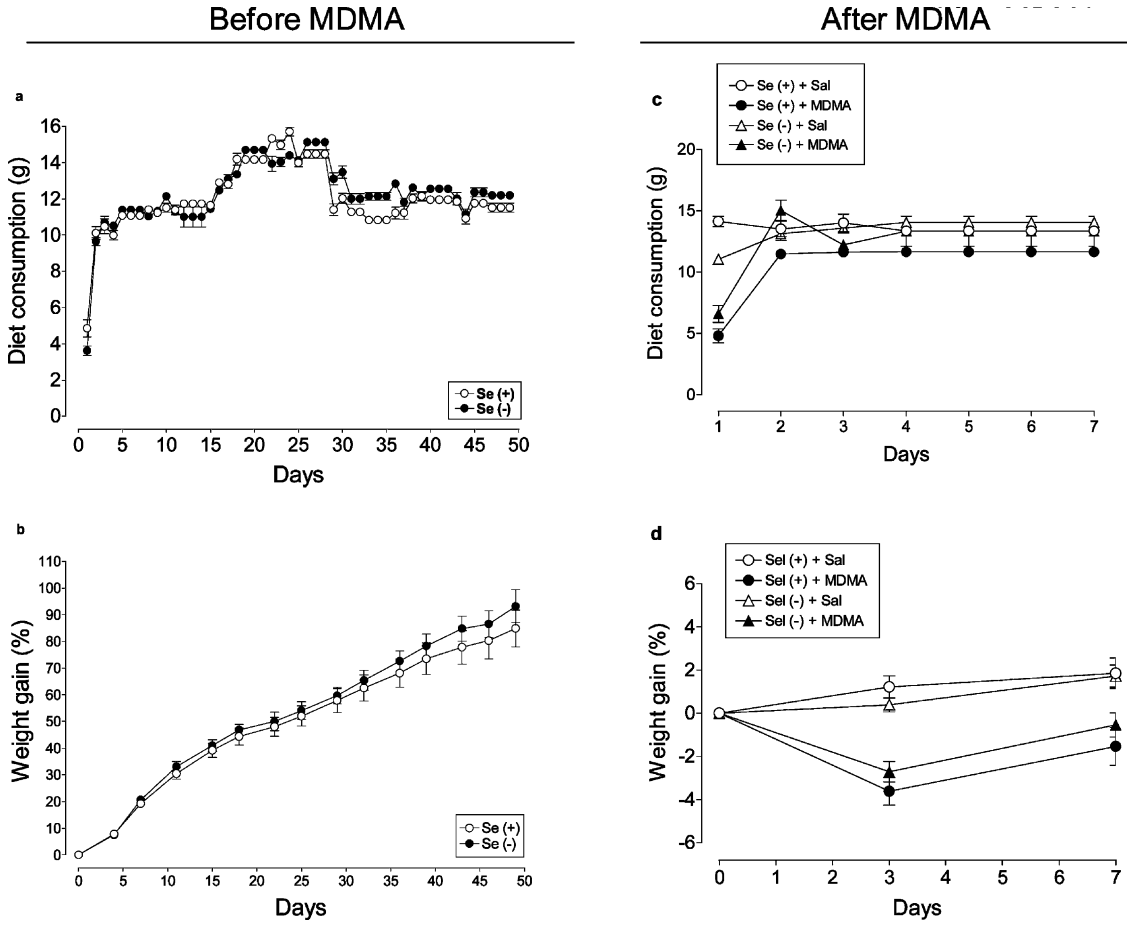

Fig. 6. Diet consumption (a,c) and increase in body weight (b,d) of rats fed either a Se-replete (Se+) or Se-deficient (Se−) diet. Animals were maintained on dietary treatment for 7 weeks (a,b). Then, each dietary group was in turn divided into two groups, which received MDMA (12.5 mg/kg, i.p.) or saline, respectively. Animals were maintained on dietary treatment for a further week before sacrificing (c,d). Results are shown as mean ± SEM (n = 16–18 before MDMA and n = 7–10 after MDMA administration). (c) MDMA decreased the diet consumed by both Se (+) and Se (−) groups compared with saline at 24 h ($P < 0.001$ for both diet groups). (d) MDMA also produced a decrease in weight gain in both the Se (+) [$F(1,13) = 24.3$, $P < 0.001$] and Se (−) [$F(1,12) = 27.8$, $P < 0.001$] groups.

Table 2
Concentrations of 5-HT and 5-HIAA in cortex, hippocampus and striatum and density of [3H]-paroxetine labeled 5-HT uptake sites in cortex of rats fed either a Se-replete (Se+) or Se-deficient (Se−) diet and given MDMA (12.5 mg/kg, i.p.)

	Diet	Saline	MDMA*
Cortex			
5-HT (ng/g tissue)	Se (+)	340 ± 17	242 ± 14 (29↓)
	Se (−)	344 ± 21	230 ± 18 (33↓)
	Standard	294 ± 11	193 ± 27 (34↓)
5-HIAA (ng/g tissue)	Se (+)	189 ± 6	133 ± 2 (30↓)
	Se (−)	185 ± 7	129 ± 8 (30↓)
	Standard	193 ± 10	141 ± 13 (27↓)
[^{3}H]-paroxetine binding (fmol/mg protein)	Se (+)	86 ± 9	32 ± 8 (63↓)
	Se (−)	80 ± 3	36 ± 4 (55↓)
	Standard	75 ± 6	38 ± 4 (49↓)
Hippocampus			
5-HT (ng/g tissue)	Se (+)	377 ± 10	186 ± 10 (51↓)
	Se (−)	352 ± 8	192 ± 8 (45↓)
	Standard	359 ± 16	174 ± 19 (52↓)
5-HIAA (ng/g tissue)	Se (+)	355 ± 11	201 ± 13 (43↓)
	Se (−)	345 ± 21	190 ± 12 (45↓)
	Standard	330 ± 30	219 ± 16 (34↓)
Striatum			
5-HT (ng/g tissue)	Se (+)	633 ± 32	425 ± 36 (33↓)
	Se (−)	670 ± 32	422 ± 21 (37↓)
	Standard	599 ± 31	418 ± 18 (30↓)
5-HIAA (ng/g tissue)	Se (+)	625 ± 46	412 ± 29 (34↓)
	Se (−)	620 ± 46	421 ± 22 (32↓)
	Standard	498 ± 24	399 ± 12 (20↓)

Animals were maintained on dietary treatment for 7 weeks before receiving MDMA and for one further week. The figure in parenthesis is the percentage of change vs. the respective control group. Results are shown as mean ± SEM (n = 7–10). Different from the corresponding saline-group: $^*P < 0.001$.

Table 3
Concentrations of DA, DOPAC and HVA in the striatum of rats fed either a Se-replete (Se+) or Se-deficient (Se−) diet and given MDMA (12.5 mg/kg, i.p.)

	Diet	Saline	MDMA
DA (ng/g tissue)	Se (+)	10190 ± 275	10243 ± 352 (1↑)
	Se (−)	10340 ± 357	9950 ± 220 (4↓)
	Standard	9731 ± 710	9854 ± 867 (1↑)
DOPAC (ng/g tissue)	Se (+)	1394 ± 78	1314 ± 22 (6↓)
	Se (−)	1437 ± 44	1291 ± 67 (10↓)
	Standard	1466 ± 76	1297 ± 76 (12↓)
HVA (ng/g tissue)	Se (+)	1577 ± 69	1310 ± 53 (17↓)
	Se (−)	1514 ± 59	1283 ± 92 (15↓)
	Standard	1658 ± 103	1578 ± 140 (6↓)

Animals were maintained on dietary treatment for 7 weeks before receiving MDMA and for one further week. The figure in parenthesis is the percentage of change vs. the respective control group. Results are shown as mean ± SEM (n = 7–10).

enhancement in malondialdehyde formation. Taken together, these findings indicate that the potentiation of MDMA toxicity on dopaminergic terminals induced by a Se deficient diet is due to an impairment of the antioxidant detoxification system mediated by glutathione. It seems reasonable to hypothesize that a decrease in the GPx activity could lead to an accumulation of H_2O_2, which might supersede the detoxification capacity of catalase. According to the Fenton reaction, the H_2O_2 in excess could react with transition metals such as iron producing a potentiation in hydroxyl radical formation (Nappi and Vass, 1997, 1998).

The involvement of oxidative stress and free radicals in MDMA-induced neurotoxicity in mice is well established. There is evidence to show that MDMA increases by 150–200% the formation of 2,3-DHBA from salicylic acid perfused through a microdialysis probe implanted in the striatum (Colado et al., 2001). This increase in

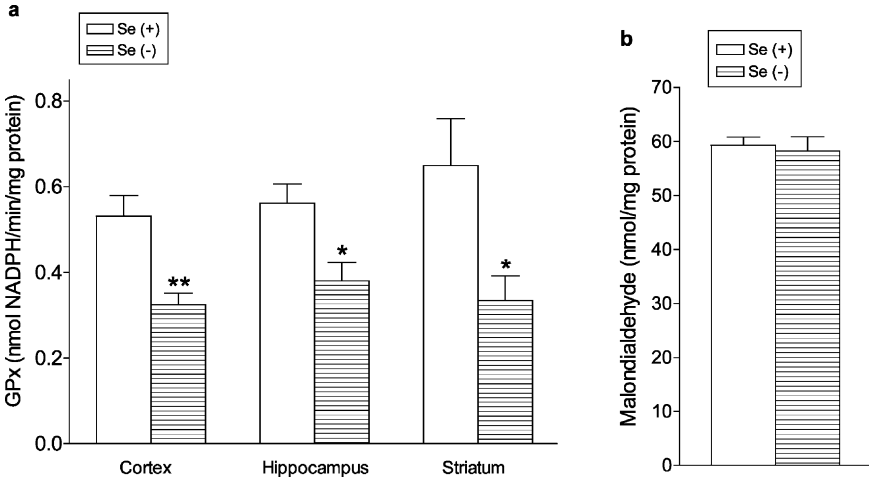

Fig. 7. GPx activity in: (a) cortex, hippocampus and striatum and lipid peroxidation; (b) cortical synaptosomes of rats fed either a Se+ or Se− diet for 8 weeks. Malondialdehyde formation in vitro was induced by incubation of synaptosomes with $FeCl_2$ (3 μM) + ascorbic acid (0.1 mM). Results are shown as mean ± SEM (n = 6–8). Different from Se-replete + saline group: $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 4
GPx and lipid peroxidation in brain regions of rats fed a standard diet

	GPx	Lipid peroxidation
Cortex	0.50 ± 0.05	50.76 ± 2.56
Hippocampus	0.48 ± 0.05	55.97 ± 1.18
Striatum	0.45 ± 0.04	54.64 ± 1.77

Results are shown as mean ± SEM mean (n = 7). GPx activity expressed as nmol NADPH formed/min/mg protein and lipid peroxidation as nmol malondialdehyde formed/mg protein.

hydroxyl radical formation induces, in turn, an increase in the degree of striatal lipid peroxidation (Camarero et al., 2002a). Selective neuronal NO inhibitors such as AR-R17477AR block both effects and prevent the long-lasting DA depletion (Colado et al., 2001). Since these compounds do not produce their neuroprotective effect by an action on MDMA-induced hyperthermia (Colado et al., 2001), it can be concluded that NO plays a role in MDMA neurotoxicity in the mouse. In addition to hydroxyl radicals and NO, there is indirect evidence indicating that the superoxide anion could also mediate long-term MDMA-induced damage in the mouse. Thus, it has been shown that MDMA-induced DA depletion is attenuated when the compound is administered to transgenic mice overexpressing CuZn SOD (Cadet et al., 1995). NO might interact with superoxide to form tissue-damaging peroxynitrite ($ONOO^-$) (Pryor and Squadrito, 1995; Imam and Ali, 2000). Peroxynitrite can be protonated to form peroxynitrous acid (ONOOH), which subsequently yields hydroxyl radicals (Beckman et al., 1990). All of these oxygen and nitrogen reactive species, as well as directly oxidizing lipid membranes, may also contribute to unbalancing antioxidant defense mechanisms. Thus, NO donors, peroxynitrite precursors and synthetic peroxynitrite all inactivate GPx by oxidation of the selenocysteine center, amino acid which is essential for peroxidase activity (Asahi et al., 1997). As GPx not only contributes to the detoxification of H_2O_2 but also plays a major role in detoxifying peroxynitrite (Sies et al., 1997), its inability to scavenge peroxides leads, in turn, to an increase in intracellular H_2O_2 and peroxynitrite and a high degree of cellular damage.

Of particular interest is the observation that a Se-deficient diet induces the appearance of long-term toxicity on 5-HT content in several brain areas of the mouse, an effect not observed either in mice maintained on an Se-replete diet or in those fed standard diet (O'Shea et al., 2001, this paper). There is evidence that repeated administration of MDMA to mice induces 16 h later, a decrease in GPx activity as well as SOD and catalase activities in several brain areas: caudate-putamen, frontal cortex and hippocampus (Jayanthi et al., 1999). These changes in enzymatic activities do not result in a toxic effect on nerve terminals containing 5-HT, which indicates that the remaining enzymatic activity is sufficient to detoxify superoxide anion and H_2O_2 from the brain probably due to a minor presence of the oxygen reactive species in 5-HT nerve terminals. Nevertheless, mice fed Se-deficient diet show a defect in the basal detoxification capabilities (as reflected by a

decrease of GPx activity not only in areas enriched in dopaminergic terminals but also in those containing 5-HT) and this effect could be the reason for the appearance of long-term 5-HT depletion induced by MDMA.

In contrast to the effects induced by MDMA in mice fed a Se-deficient diet, the administration of this drug to mice maintained with a Se-replete diet (0.2 ppm) or a standard diet (0.16 ppm) only produces a long-term loss of striatal DA concentration leaving intact 5-HT containing neurons. This different susceptibility of dopaminergic and serotonergic neurons to the neurotoxic damage of MDMA has been previously described (Stone et al., 1987; Battaglia et al., 1988; Logan et al., 1988; O'Callaghan and Miller, 1994; O'Shea et al., 2001) and could be attributed to the fact that the brain detoxification system against free radicals is less efficient in dopaminergic than in serotonergic neurons. In fact, our results, support this hypothesis since in spite of GPx activity tending to be higher (although not significantly) in the striatum than in the cortex, the degree of $FeCl_2$ + ascorbic acid-induced lipid peroxidation was also more pronounced. These data indicate that an increase in GPx activity does not necessarily result in a higher detoxificant effect but also that the GPx activity is probably related to the need to detoxify H_2O_2 which is continuously formed in the course of striatal DA metabolism (Brannan et al., 1980; Somani et al., 1995).

Mice maintained on an Se-deficient diet (<0.02 ppm) showed a smaller weight gain over time than the Se-replete animals, indicating that Se is a micronutrient essential for optimum growth and that the requirement of dietary Se in mice is greater than that contained in the deficient diet. In fact the National Research Council sets the dietary Se requirement at 0.1 mg Se/kg diet (=0.1 ppm). An attenuated weight gain in mice produced by Se depletion has been described previously (Kim et al., 1999). The reduction in the growth produced by Se deficiency is not due to a decreased diet consumption (this paper), so it is reasonable to propose that malabsorption of nutrients might be one of the reasons for decreased weight gain. Moreover, long-lasting Se depletion decreases the activity of thioredoxin reductase, an enzyme which is involved in regulating cell growth through its reduction of thioredoxin (Berggren et al., 1999).

What does appear clear is that the differences observed in MDMA toxicity between mice fed a Se-deficient or Se-replete diet are not due to an effect related to body temperature. Although the hyperthermic response induced by the second and third injections of MDMA in Se-deficient animals was slightly higher than in Se-replete mice, the area under the curve was not statistically different between the two groups.

Unlike mice, where the Se-deficient diet exacerbated the MDMA-induced long-term neurotoxicity, no change was observed in the long-term loss of tissue 5-HT content following MDMA administration to rats. Thus, animals maintained with either a Se-deficient or Se-replete diet for 8 weeks showed a similar reduction in both the indole concentration and the density of 5-HT uptake sites in the brain. In spite of the lack of effect of dietary treatment on MDMA damage, rats fed a Se-deficient diet did show a reduction in GPx activity, compared with those fed a Se-replete diet. Nevertheless, this reduced ability of the glutathione system to detoxify peroxides did not lead to an increased oxidative stress since the cortical synaptosomes of rats maintained with a Se-deficient diet showed the same degree of lipid peroxidation following a stressant stimulus such as $FeCl_2$ + ascorbic acid. These observations indicate that a reduction in the GPx activity in the rat does not lead to a decrease in the ability of rat brain to detoxify hydrogen peroxide and suggest that the capacity of the catalase system, which has very low activity but is widely distributed in the rat brain, is sufficient to compensate for the loss of GPx activity (Gaunt and de Duve, 1976; Brannan et al., 1981). In addition, there is evidence that the amount of free radicals formed in rat brain following MDMA is considerably less than that found in mouse brain. If we compare the formation of 2,3-DHBA in the brain of mice and rats it can be seen that the increase of this compound in the hippocampal dialysate of the rat (50%) is substantially less than that observed in the striatal dialysate of the mouse (180–200%) (Colado et al., 1997a, 2001), which indicates that a neurotoxic dose of MDMA induces a relatively modest rise in hydroxyl radical formation in the rat brain. On the other hand, in contrast to mice, there is no clear evidence of the involvement of NO in MDMA-induced neurotoxicity in rats. We have been unable to demonstrate that administration of selective neuronal NOS inhibitors provide protection against damage in rats (unpublished observations) and the only data showing some neuroprotective effect of NOS inhibitors resulted from the administration of compounds with poor selectivity for neuronal NOS (Zheng and Laverty, 1998). Therefore, it is reasonable to propose that cells producing high levels of different species of free radicals, as occurring in mouse brain, are especially vulnerable to oxidative stress and therefore, respond to an insufficient detoxification of reactive oxygen species via the glutathione system.

In contrast to the effect in mice, an Se-deficient diet did not reduce the normal weight gain in rats. This observation has previously been reported (Meydani et al., 1988; Lei et al., 1995) and could be attributed to the fact that activity of the selenoenzyme is less sensitive to Se dietary content in rats compared with mice. Thus, rats maintained for 14 weeks with an Se-deficient diet did not show any change in the brain activity of thioredoxin reductase (Hill et al., 1997).

In summary, this study shows that Se deficiency impairs the cellular antioxidant status of the mouse brain and that this effect not only exacerbates the dopami-

nergic toxicity induced by MDMA but also facilitates the appearance of damage to 5-HT containing neurons. In contrast, rats fed an Se-deficient diet do not show any change in MDMA-induced neurotoxicity of 5-HT nerve terminals in spite of showing a decrease in brain GPx activity.

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