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24

The Effects of Aminorex and Related Compounds on Brain Monoamines and Metabolites in CBA Mice

YIWEN ZHENG, BRUCE RUSSELL, DAVID SCHMIERER* AND RICHARD LAVERTY

*Department of Pharmacology and *School of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand*

Abstract

Acute and long-term neurochemical effects of aminorex, an appetite-suppressing drug related to amphetamine in chemical structure, and stereoisomers of its analogues were examined and compared with those of 3,4-methylenedioxymethylamphetamine (MDMA) and fenfluramine.

Aminorex and its analogues, with exception of 4*S*, 5*S*-dimethylaminorex, did not cause the long-term neurotransmitter depletion in either the dopaminergic or 5-HT-ergic systems that was observed after MDMA or fenfluramine in CBA mice. These results are discussed in terms of possible structurally related mechanisms of neurotoxicity.

The acute neurochemical effects showed that aminorex and analogues all produced increases in 5-hydroxytryptamine (5-HT) levels, unlike fenfluramine and MDMA in the present study or in published data. This suggests that inhibition of 5-HT metabolism, rather than direct 5-HT release, may be involved in their anorectic effect. The parallel study of acute dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) changes suggest that in CBA mice MDMA may be a better dopamine releaser and this may contribute to its dopaminergic neurotoxicity. However the ability to release dopamine or 5-HT, or both, may be important, but not the only factor involved in causing the long-term neurotoxicity observed with amphetamine derivatives.

Aminorex (2-amino-5-phenyl-2-oxazoline) is an appetite suppressant used for slimming which was removed from the market in Europe in 1968 amid reports that it induced chronic pulmonary hypertension and consequent death (Gurtner et al 1968). Aminorex is related to amphetamine in chemical structure and was reported to possess the anorectic properties of amphetamine (Roszkowski & Kelley 1963). Drug discrimination studies have shown that aminorex produces stimulus effects similar to amphetamine but not to fenfluramine (Young 1992). In recent years, 4-methylaminorex, which is similar in chemical structure to aminorex, has been confiscated by drug control authorities in Florida, California and Pennsylvania (Davis & Brewster 1988; Drug Control Section 1988). Although both the *cis* and *trans* isomers of 4-methylaminorex are purported to have similar stimulant and anorectic properties comparable with those of amphetamine (Roszkowski & Kelley 1963; Yelnosky & Katz 1963), only the *cis* isomer of 4-methylaminorex was classified in the USA as a Schedule I substance in April 1989. In our laboratory, previous studies with aminorex and its methyl- or dimethyl- analogues have shown that these compounds produced effective inhibition of the food consumption in food-deprived animals (Ram & Laverty 1993). Although these agents appear to cause stimulant and anorectic effects, little has been published about their pharmacological and toxicological properties.

Amphetamine and many of its analogues have appetite-suppressing properties and also produce long-term neurotoxicity in rats, mice and monkeys after repeated high-dose treatment (Clineschmidt et al 1976; Schmidt et al 1986; Ricaurte et al 1988). These structurally related compounds show different selectivity in long-term neurotoxicity. For example, in rats fenfluramine and methylenedioxymethylamphetamine (MDMA) appear to attack preferentially the 5-hydroxytryptaminergic (5-HT-ergic) system, methamphetamine attacks both the 5-HT-ergic and dopaminergic system, while amphetamine itself only attacks the dopaminergic system (Clineschmidt et al 1976; Steranka & Sanders-Bush 1979; Schmidt et al 1986; Schmidt 1987). Although it is well accepted that MDMA acts as a selective 5-HT-ergic neurotoxin in rats, we found that in CBA mice it attacked both the 5-HT-ergic and dopaminergic systems as indicated by long-term 5-HT and dopamine depletion (Logan et al 1988; Zheng & Laverty 1993). Structure-activity relationship studies indicate that the stereoisomers of amphetamine, MDMA and fenfluramine have different potency and selectivity in monoamine release, inhibition of reuptake or neurotoxicity (Johnson et al 1986; Steele et al 1987; Johnson & Nichols 1989; Nichols et al 1989; Johnson & Nichols 1990). Furthermore, a number of studies suggest that acute release of dopamine or 5-HT, or both, might be involved in the long-term neurotoxicity of amphetamine analogues (Stone et al 1988; Axt et al 1990). These observations led us to consider whether these stereoisomers of aminorex would produce long-term neurotoxicity in mice and whether the acute neurochemical effect produced by these aminorex compounds and other amphetamines are different. We therefore examined the long-term and acute neurochemical effects of aminorex and the stereoisomers, 4*R*,5*S*-methylaminorex, 4*S*,5*S*-methylaminorex, 4*R*,5*R*-methylaminorex, 4*R*,5*S*-dimethylaminorex and 4S,5S-dimethylaminorex (Fig. 1, see the chemical structure), after repeated or single treatment in CBA mice, compared with those after MDMA and fenfluramine.

Correspondence: Y. Zheng, Department of Pharmacology. Medical School, University of Otago, P.O. Box 913, Dunedin, New Zealand. E-mail: yiwen.zheng@stonebow.otago.ac.nz

Materials and Methods

Animal treatment and dissection

CBA mice (male, 20–25 g, from the Animal Breeding Station, University of Otago) were housed in temperature-controlled rooms with a 12-h alternating light/dark cycle and allowed free access to standard laboratory food and water.

For long-term neurochemical effect, groups of mice (n = 10) were injected intraperitoneally with saline, MDMA (25 mg kg^{-1}), fenfluramine (25 mg kg^{-1}), aminorex (25 mg kg^{-1}), 4*R*,5*S*-methylaminorex (15 mg kg^{-1}), 4*S*,5*S*-methylaminorex (15 mg kg^{-1}), 4*R*,5*R*-methylaminorex (15 mg kg^{-1}), 4*R*,5*S*-dimethylaminorex (30 mg kg^{-1}) or 4*S*,5*S*-dimethylaminorex (5 mg kg^{-1}) respectively. The doses were chosen from acute toxicity experiments (data not shown) to be the maximum tolerated with little or no lethality. The dosages used in the present experiments are about 6–10 times higher than the effective doses of aminorex and stereoisomers for the inhibition of food intake. Doses were repeated 3 times in a 24 h period (usually 17:00, 09:00, 17:00). Mice were killed by cervical dislocation 7 days after the last dose. For the acute effects, a single dose was given to each group and the mice were killed at 1, 3 or 6 h after administration. For long-term effects, samples of striatum and for acute effects, samples of striatum, cortex and hippocampus were removed and immediately frozen on dry ice. Tissue samples were stored at −80°C until assayed.

Determination of monoamines and metabolites of monoamines

Tissue concentrations of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) were determined by high performance liquid chromatography with electrochemical detection as in a previous study (Zheng & Laverty 1993). Briefly, samples were homogenised in 0·4 M $HClO_4$ and centrifuged at 4°C. The supernatant (20 μL) was injected onto a 100 × 4·6 mm, 5 μm, C_8, MOS column and eluted with a mobile phase containing 10% (v/v) acetonitrile, 100 mM NaH_2PO_4, 1 mM octylsulphonic acid, 19·5 mM sodium acetate and 0·3 mM Na_2EDTA at pH 2·6. The flow rate was set to 1·5 mL min^{-1}. The ESA model 5100A Coulochem electrochemical detection system consisted of a model 5020 guard cell (detector setting, +1·04 V), followed in sequence by a model 5010 analytical cell (detector 1, +0·3 V, detector 2, +0·45 V). Components were quantified by comparison with a curve generated from external standards.

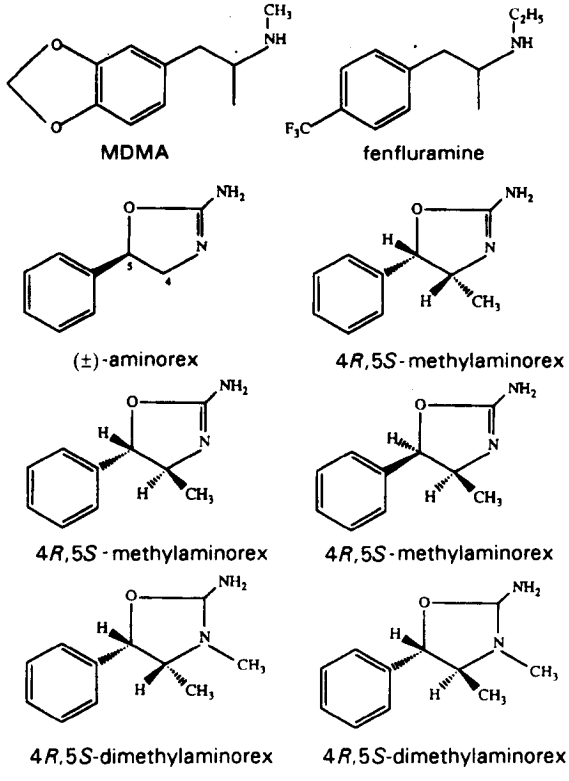

FIG. 1. The chemical structure of MDMA, fenfluramine and aminorex analogues used in the experiment.

Drugs

The following drugs were synthesized in the School of Pharmacy, University of Otago: MDMA, aminorex, 4*S*,5*S*-methylaminorex, 4*R*,5*R*-methylaminorex, 4*R*,5*S*-methylaminorex, 4*S*,5*S*-dimethylaminorex and 4*R*,5*S*-dimethylaminorex. Fenfluramine was purchased from Sigma Chemical Co. Aminorex and 4*S*,5*S*-methylaminorex were dissolved by a drop of 60% H_2SO_4 and adjusted to pH 6·0 with Na_2HPO_4. The other drugs all dissolved in saline. Saline was used as vehicle control.

Statistical analysis

The results are presented as the mean ± s.e.m. of the tissue contents of the treatment groups expressed as a percentage of corresponding saline-injected controls for ease of comparison.

Table 1. Effects of fenfluramine, MDMA and aminorex analogues (3 doses in 24 h) on striatal levels (% control) of monoamines and metabolites in CBA mice 7 days after the final day administration.

Drugs	Dose (mg kg^{-1})	Level of monoamines and metabolites (% control)			
		DOPAC	Dopamine	5-HIAA	5-HT
MDMA	25	70.8 ± 5.7**	79.7 ± 4.8**	78.6 ± 6.1*	77.2 ± 7.9*
Fenfluramine	25	88.0 ± 6.7	98.5 ± 5.8	73.9 ± 4.3**	77.2 ± 10.4**
Aminorex	25	100.5 ± 3.5	97.2 ± 3.7	98.0 ± 2.6	99.0 ± 5.3
4*R*,5*S*-Methylaminorex	15	123.7 ± 3.2**	99.6 ± 3.6	101.7 ± 2.0	102.0 ± 2.9
4*S*,5*S*-Methylaminorex	15	138.0 ± 4.6**	105.8 ± 4.5	99.7 ± 3.0	96.9 ± 3.7
4*R*,5*R*-Methylaminorex	15	133.0 ± 3.2	100.1 ± 3.3	98.5 ± 3.0	91.9 ± 6.85
4*R*,5*S*-Dimethylaminorex	30	124.5 ± 4.3**	102.4 ± 6.8	104.6 ± 4.1	99.8 ± 7.9
4*S*,5*S*-Dimethylaminorex	5	105.8 ± 3.5	86.5 ± 3.7**	105.0 ± 3.2	94.6 ± 6.0

Results are the mean ± s.e.m. from 8–10 brains. *$P < 0.05$, **$P < 0.01$, significantly different from control by one-way analysis of variance followed by Student-Newman-Keuls multiple comparison.

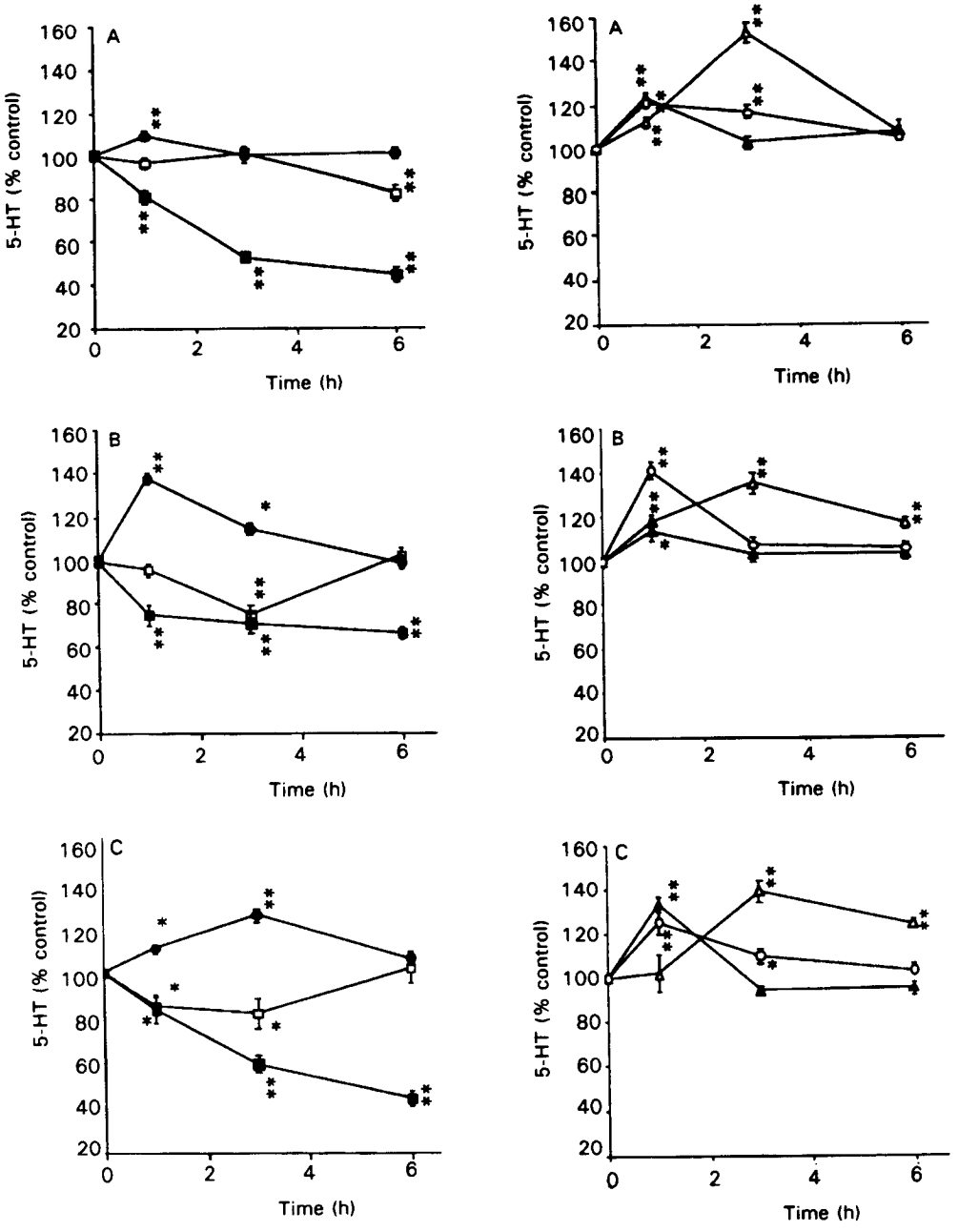

FIG. 2. Acute effect on 5-HT levels (% control) in cortex (A), hippocampus (B) and striatum (C) of CBA mice after a single intraperitoneal dose. □ MDMA (50 mg kg^{-1}), ■ fenfluramine (25 mg kg^{-1}), ● aminorex (25 mg kg^{-1}), △ 4*S*,5*S*-methylaminorex (15 mg kg^{-1}), ▲ 4*S*,5*S*-dimethylaminorex (5 mg kg^{-1}) or ○ 4*R*,5*S*-dimethylaminorex (30 mg kg^{-1}). Data are presented as the mean percent change (± s.e.m.; n = 10) from control. Differences between drug and saline treatments were determined by one-way analysis of variance followed by Student-Newman-Keuls multiple comparison. $*P < 0.05$, $**P < 0.01$.

Comparisons were made by one-way analysis of variance. Where a significant effect of drug occurred, Student-Newman-Keuls multiple comparisons were carried out.

Results

Long-term neurochemical effects

Table 1 shows the monoamine levels in striatum 7 days after drug administration. MDMA (25 mg kg^{-1}) reduced the concentrations of dopamine, 5-HT and their metabolites, while fenfluramine (25 mg kg^{-1}) only caused a marked depletion of 5-HT and 5-HIAA but no effect on the dopaminergic system. These results agree with previous studies (Logan et al 1988; Zheng & Laverty 1993). The stereoisomers of methylaminorex and 4*R*,5*S*-dimethylaminorex produced a significant increase of DOPAC, but aminorex and 4*S*,5*S*-dimethylaminorex had no effect on DOPAC level. In addition, there were no changes in dopamine, 5-HT and 5-HIAA levels in striatum except that a decrease of dopamine concentration was caused by 4*S*,5*S*-dimethylaminorex.

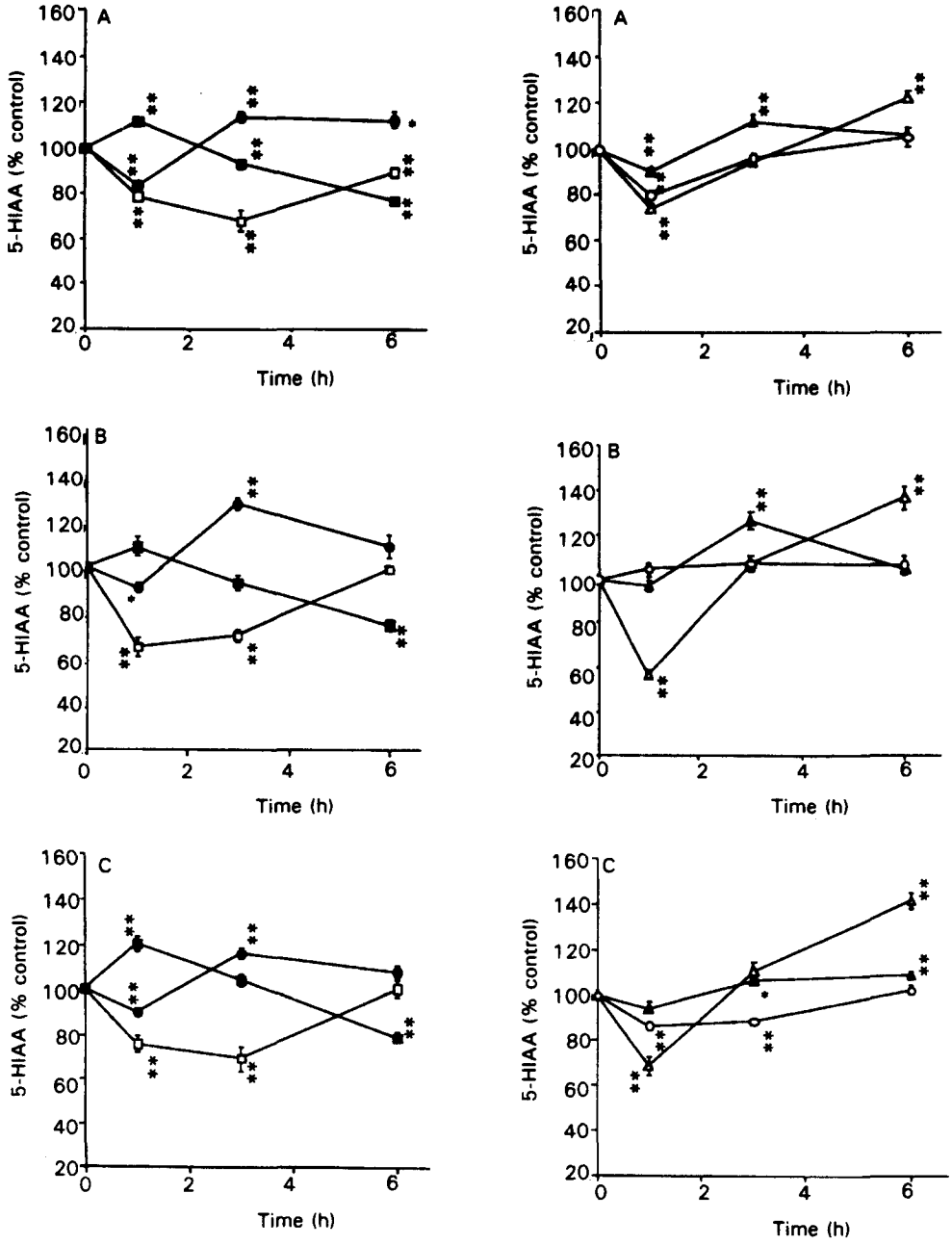

FIG. 3. Acute effect on 5-HIAA level (% control) in cortex (A), hippocampus (B) and striatum (C) of CBA mice after a single intraperitoneal dose. □ MDMA (50 mg kg^{-1}), ■ fenfluramine (25 mg kg^{-1}), ● aminorex (25 mg kg^{-1}), △ 4*S*,5*S*-methylaminorex (15 mg kg^{-1}), ▲ 4*S*,5*S*-dimethylaminorex (5 mg kg^{-1}) or ○ 4*R*,5*S*-dimethylaminorex (30 mg kg^{-1}). See Fig. 2 for additional details.

Acute neurochemical effects

As shown in Fig. 2, a single dose of fenfluramine caused a significant decrease in 5-HT levels during the period of the experiment in all three brain regions. MDMA had a similar effect but the 5-HT levels returned to normal in hippocampus and striatum at 6 h. Aminorex and its analogues produced an increase in 5-HT concentration initially but this returned towards the control level by 6 h.

The corresponding changes in 5-HIAA levels are shown in Fig. 3. Fenfluramine increased 5-HIAA at 1 h followed by a significant decrease. MDMA reduced 5-HIAA concentrations at 1 and 3 h but levels returned towards the control level by 6 h except that 5-HIAA remained low in cortex at 6 h. Aminorex and analogues produced a decrease of 5-HIAA levels at 1 h followed by an increase to the control levels or even higher.

In striatum, fenfluramine and MDMA decreased dopamine levels at the first hour after administration and then increased it towards or beyond normal (Fig. 4). Aminorex caused an increase in dopamine levels at 1 h which remained high for the duration of the experiment (Fig. 4). 4*S*,5*S*-methylaminorex caused no change of dopamine levels by 3 h, however there was a marked increase at 6 h. While both dimethyl analogues

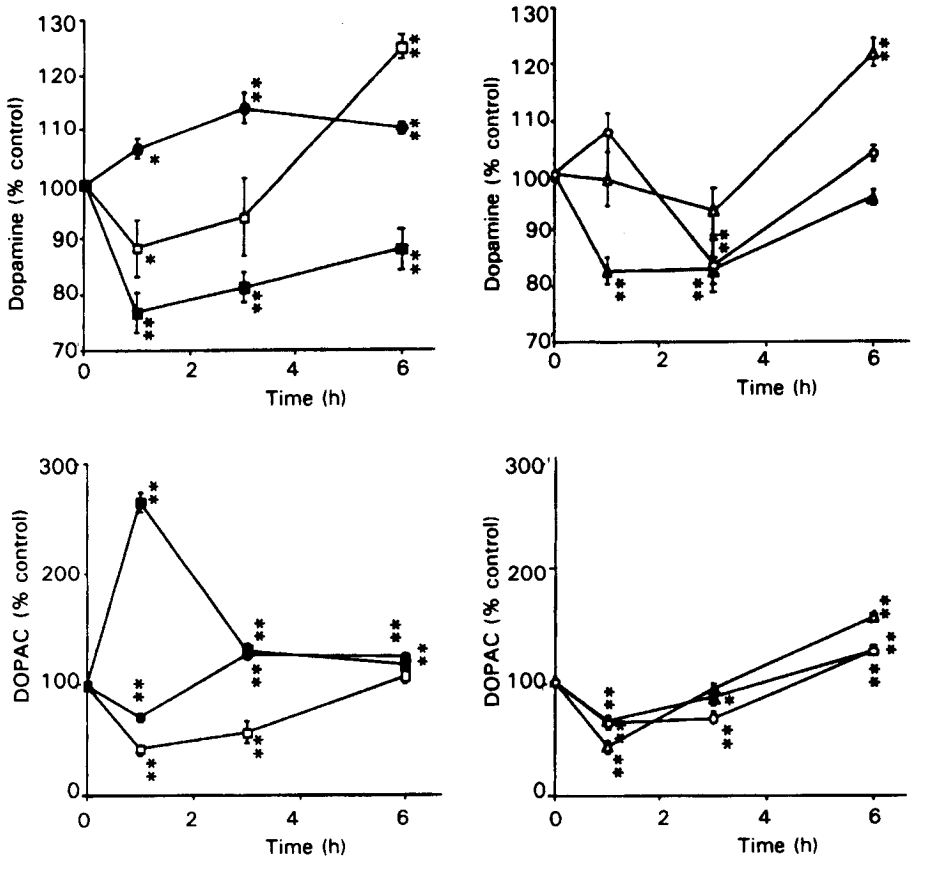

FIG. 4. Acute effect on dopamine and DOPAC levels (% control) in striatum of CBA mice after a single intraperitoneal dose. □ MDMA (50 mg kg^{-1}), ■ fenfluramine (25 mg kg^{-1}), ● aminorex (25 mg kg^{-1}), △ 4*S*,5*S*-methylaminorex (15 mg kg^{-1}), ▲ 4*S*,5*S*-dimethylaminorex (5 mg kg^{-1}) or ○ 4*R*,5*S*-dimethylaminorex (30 mg kg^{-1}). See Fig. 2 for additional details.

of aminorex induced significant reduction of dopamine by 3 h, the levels return to normal at 6 h (Fig. 4). Except for fenfluramine which induced a marked DOPAC increase at 1 h in striatum, the other drugs all decreased DOPAC at first and increased it later (Fig. 4).

Discussion

In the first part of this study we examined the long-term neurochemical effects of aminorex and its five analogues. The long-term neurochemical changes after MDMA and fenfluramine are used as a positive control of dopaminergic or 5-HT-ergic neurotoxicity, or both, induced by amphetamines. Since a long-lasting depletion of dopamine or 5-HT appears to be a good predictor of dopamine or 5-HT neurotoxicity (Wagner et al 1980; Ricaurte et al 1985), the results suggest that the aminorex compounds except 4*S*,5*S*-dimethylaminorex, unlike MDMA or fenfluramine, are not toxic to either dopamine or 5-HT neurotransmitter systems in CBA mice. It was reported that although multiple doses of 4-methylaminorex caused long-term (7 day) declines in striatal tryptophan hydroxylase activity in SD rats, no changes were found in 5-HT and 5-HIAA levels (Hanson et al 1992). Our findings generally agree with previously published data and extend the observations that have been made with different isomers of aminorex.

Much of the work with amphetamine and its analogues suggests that varying the identity of substituent groups on the ethylamine side chain alters the potency and ability of these compounds to elicit stimulatory, psychotomimetic and neurotoxic effects. For example, the inhibitory potency on [^{3}H] dopamine uptake by striatal synaptosomes progressively diminishes with increasing substitution of the *S*-(+)-enantiomers [*S*-(+)-amphetamine > *S*-(+)-3,4-methylenedioxyamphetamine (MDA) > *S*-(+)-MDMA > *S*-(+)-*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB)] (Steele et al 1987); the neurotoxic effect of MBDB on the 5-HT-ergic system is less than that of MDMA (Johnson & Nichols 1989). Comparison of the psychotomimetic potency of the one-ring psychotomimetics shows that there is also a decrease in activity with the introduction of an alpha methyl group (Shulgin et al 1969). Ricaurte et al (1989) demonstrated the neurotoxicity of *N*,*N*-dimethylamphetamine; their results suggest that *N*-methylation markedly attenuates the neurotoxic potency of methamphetamine. Furthermore, the rigid analogue 5,6-methylenedioxy-2-aminoindan (MDAI) is devoid of any long-term effects on the 5-HT-ergic system after a single dose of up to 40 mg kg^{-1} (Nichols et al 1990). Therefore, it seems that increasing the length of the side chain, *N*-methylation and

cyclization of the ethylamine side chain could attenuate or abolish the neurotoxic effects of amphetamine analogues. In our results, aminorex and analogues all have a large and cyclized substitution on the nitrogen, and have no significant neurotoxicity. It suggests that the stability of ethylamine side chain may be involved in the mechanisms of inducing long-term neurotoxicity by amphetamine and analogues. It is interesting that 4*S*,5*S*-dimethylaminorex, which has similar basic chemical structure and acute neurochemical effects as other aminorex compounds, causes long-term dopamine depletion. It should be mentioned that the dose of 4*S*,5*S*-dimethylaminorex is 3 times less than that of methylaminorex and 6 times less than that of 4*R*,5*S*-dimethylaminorex because of its ability to produce severe, fatal seizures. Appetite studies have shown that 4*S*,5*S*-dimethylaminorex is the most effective agent in inhibition of food consumption among these isomers in CBA mice and the ED50 of this drug is significantly less than that of fenfluramine (unpublished data). As we discussed before, the ethylamine side chain makes these aminorex isomers less neurotoxic than amphetamine analogues. However, according to the established structure-activity relationships for central stimulant and discriminative stimulus properties, the potency associated with amphetamines that have an α-methyl group in the *S*- configuration is greater than the *R*- configuration. Therefore, it would be expected that the 4*S*- isomers of methylaminorex or dimethylaminorex are more potent than the 4*R*- isomers. In addition, dimethylaminorex has an *N*-methyl substituted terminal amine which is more structurally related to methamphetamine. Since methamphetamine is said to be more potent than amphetamine, dimethylaminorex would be expected to have a greater potency than methylaminorex. These factors may explain why 4*S*,5*S*-dimethylaminorex is the only isomer to produce long-term dopamine depletion among the aminorex isomers tested.

It has been suggested that MDMA neurotoxicity could have resulted from the in-vivo formation of a metabolite because of the absence of neurotoxicity after direct administration into the brain (Paris & Cunningham 1991). The metabolites of MDMA may be produced by *N*-demethylation, *O*-dealkylation, aromatic hydroxylation, deamination or conjugation of MDMA and its metabolites (Lim & Foltz 1988, 1991a,b). Therefore, for aminorex compounds, perhaps the cyclized *N*-substitution with a ring double bond could increase stability and interfere with formation of such a metabolite.

In-vitro and in-vivo studies have demonstrated that fenfluramine causes 5-HT release in brain (Mennini et al 1985; Schwartz et al 1989). The decrease of 5-HT and 5-HIAA levels observed following fenfluramine injection are thought to be a result of 5-HT release (see review by Rowland & Carlton 1986). In our results, fenfluramine induces a reduced 5-HT level throughout the 6 h period of experiment (Fig. 2). Brain 5-HIAA levels are increased at first (1 h) and then decreased at 3 and 6 h (Fig. 3). Fuller et al (1978) reported that 5-HIAA depletion lags behind that of 5-HT depletion and suggests that inhibition of monoamine oxidase is probably of no importance in fenfluramine's action. However, the initial increase of 5-HIAA in our results might suggest that apart from its ability to release 5-HT, fenfluramine induces a rapid metabolism of 5-HT by monoamine oxidase in CBA mice which is paralleled by the dramatic increase of DOPAC 1 h after fenfluramine administration (Fig. 4).

It is believed that the acute depletion of 5-HT and 5-HIAA by MDMA is due to a marked increase in 5-HT release with a rapid decrease in the activity of tryptophan hydroxylase and an inhibition of monoamine oxidase activity (Schmidt & Taylor 1987; Schmidt et al 1987). The 5-HT and 5-HIAA changes observed in our experiments in mice after MDMA (Fig. 2) show some differences from those in rats. It is reported that in rats the decrease of 5-HIAA is behind that of 5-HT as indicated by a marked reduction after 2 h (Stone et al 1987). However we found an immediate decrease of 5-HIAA after MDMA. It suggests that MDMA induces monoamine oxidase inhibition in CBA mice and this effect seems more rapid and efficient than that in rats. The difference in the effect on 5-HT and 5-HIAA levels between fenfluramine and MDMA suggests that except for the similar release of 5-HT, fenfluramine and MDMA might affect 5-HT metabolism in different ways, although the significance of this difference is presently unclear.

In the present study, the acute effects of 5-HT and 5-HIAA after aminorex and analogues show different patterns from that of either fenfluramine or MDMA (Figs 2 and 3). Though there are differences in peak time and potency among these drugs, they all produce an increase of 5-HT and a decrease of 5-HIAA at first. It is reported that after giving the monoamine oxidase inhibitor pargyline, an increase of 5-HT concentration and a decrease of 5-HIAA concentration are found in brain (Tozer et al 1966; Neff & Tozer 1968). Therefore, our observation may suggest a reduction of 5-HT metabolism resulting from monoamine oxidase inhibition. Previous studies in our laboratory have shown that aminorex and analogues produce an effective anorectic effect in rats and mice (Ram & Laverty 1993). Although appetite is under multiple influences (Blundell 1991), particular attention has been paid to the role of 5-HT. It has been accepted that the primary mechanism of fenfluramine anorexia is via increased brain 5-HT activity (see review by Rowland & Carlton 1986). Thus if aminorex analogues produce their anorectic effect through 5-HT mechanisms, it is noteworthy that they might inhibit 5-HT metabolism rather than directly release 5-HT. Furthermore, the enhanced 5-HT function after these compounds is not accompanied by the long-term 5-HT depletion which occurs after MDMA and fenfluramine (Table 1).

Studies with amphetamines indicate a correlation between their neurotoxic spectrum and monoamine release (see review by Schmidt & Kehne 1990). In rats, amphetamine is a potent dopamine releaser but is a relatively poor 5-HT releasing agent and acts as a "pure" dopaminergic neurotoxin. Methamphetamine is similar to amphetamine in terms of dopamine release but is a considerably better 5-HT releaser and can damage both 5-HT-ergic and dopaminergic terminals. MDMA and *p*-chloroamphetamine are less effective at dopamine release but are potent 5-HT releasers and are pure 5-HT-ergic neurotoxins. This suggests that the patterns of neurotransmitter release may play a role in determining the patterns of neurotoxicity. MDMA is a moderately active releaser of dopamine (Schmidt et al 1987; Johnson et al 1991) and a monoamine oxidase inhibitor (Schmidt et al 1987) and it is reported that dopamine was significantly elevated and DOPAC declined in rats after MDMA (Stone et al 1987). In contrast, there was a marked reduction in both dopamine and DOPAC levels after MDMA (Fig. 4). It suggests that MDMA might be more effective at dopamine release in CBA mice than in rats. This may be

related to the greater dopaminergic neurotoxicity induced by MDMA in CBA mice. In our present experiments, aminorex and its methyl and dimethyl analogues produce dopamine and DOPAC changes which are similar to those after MDMA in CBA mice. However, except for 4*S*,5*S*-dimethylaminorex which causes long-term dopamine depletion, others do not have long-term effects on neurotransmitter levels in either 5-HT-ergic or dopaminergic systems (Table 1). This suggests that increase of dopamine release may be an important, but not a unique factor in determining the long-term neurotoxicity of amphetamines.

In summary, this is the first time the acute and long-term neurochemical effects of these stereoisomers of aminorex have been systematically compared with those of fenfluramine and MDMA. The results indicate that aminorex and analogues, except 4*S*,5*S*-dimethylaminorex, do not cause long-term dopamine or 5-HT depletion, or both, like those induced by either MDMA or fenfluramine in CBA mice and suggest that the property of *N*-substitution of amphetamine analogues and the 4- and 5- positions of the aminorex isomers could be the important factors associated with the ability of these compounds to induce long-term neurotoxicity in-vivo. The acute increase of 5-HT and decrease of 5-HIAA levels following aminorex compounds suggests that they may enhance 5-HT function by an inhibition of 5-HT metabolism, which probably are responsible for their anorectic effects. In contrast with the acute effect on dopamine in rats, MDMA significantly decreases both striatal dopamine and DOPAC in CBA mice in the present study, which suggests that MDMA might be a better dopamine releaser in CBA mice, so that it induces long-term neurotoxicity in both dopaminergic and 5-HT-ergic systems in this species. However, considering the similar acute, but different long-term effects produced by aminorex compounds, these results suggest that dopamine release may be an important, but not the only factor in determining the long-term neurotoxicity of amphetamines and that the mechanism(s) are more complicated than we expected and require further study.

Acknowledgement

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References

Axt, K. J., Commins, D. L., Vosmer, G., Seiden, L. S. (1990) α-Methyl-*p*-tyrosine pretreatment partially prevents methamphetamine-induced endogenous neurotoxin formation. Brain Res. 515: 269–276

Blundell, J. (1991) Pharmacological approaches to appetite suppression. Trends Pharmacol. Sci. 12: 147–157

Clineschmidt, B. V., Totaro, J. A., Mcguffin, J. C., Pflueger, A. B. (1976) Fenfluramine: long-term reduction in brain serotonin (5-hydroxytryptamine). Eur. J. Pharmacol. 35: 211–214

Davis, F. T., Brewster, M. E. (1988) A fatality involving U4Euh, a cyclic derivative of phenylpropanolamine. J. Forensic Sci. 33: 549–553

Drug Control Section, Office of Diversion Control, Drug Enforcement Admin., USA (1988) Scheduling recommendation for 4-methylaminorea, September 1988

Fuller, R. W., Snoddy, H. D., Hemrick, S. K. (1978) Effects of fenfluramine and norfenfluramine on brain serotonin metabolism in rats. Proc. Soc. Exp. Biol. Med. 157: 202–205

Gurtner, H.P., Gertsch, M., Salzmann, C., Scheerer, M., Slucki, P., Wyss, F. (1968) Hëufen sich die Primër vasculëren formen des chronischen cor pulmonale? Schweiz. Med. Wochen. 98: 1579 and 1695

Hanson, G. R., Bunker, C. F., Johnson, M., Bush, L., Gibb, J. W. (1992) Response of monoaminergic and neuropeptide systems to 4-methylaminorex: a new stimulant of abuse. Eur. J. Pharmacol. 218: 287–293

Johnson, M. P., Nichols, D. E. (1989) Neurotoxic effects of the alpha-ethyl homologue of MDMA following subacute administration. Pharmacol. Biochem. Behav. 33: 105–108

Johnson, M. P., Nichols, D. E. (1990) Comparative serotonin neurotoxicity of the stereoisomers of fenfluramine and norfenfluramine. Pharmacol. Biochem. Behav. 36: 105–109

Johnson, M. P., Hoffman, A. J., Nichols, D. E. (1986) Effects of the enantiomers of MDA, MDMA and related analogues on [^{3}H]serotonin and [^{3}H]dopamine release from superfused rat brain slices. Eur. J. Pharmacol. 132: 269–276

Johnson, M. P., Conarty, P. F., Nichols, D. E. (1991) [^{3}H]Monoamine releaseing and uptake inhibition properties of 3,4-methylenedioxymethamphetamine and *p*-chloroamphetamine analogues. Eur. J. Pharmacol. 200: 9–16

Lim, H. K., Foltz, R. L. (1988) In vivo and vitro metabolism of 3,4-(methylenedioxy)methamphetamine in the rat: identification of metabolites using an ion trap detector. Chem. Res. Toxicol. 1: 370–378

Lim, H. K., Foltz, R. L. (1991a) In vivo formation of aromatic hydroxylated metabolites of 3,4-(methylenedioxy)methamphetamine in the rat: identification by ion trap ms/ms and ms/ms/ms techniques. Biol. Mass. Spectrom. 20: 667–686

Lim, H. K., Foltz, R. L. (1991b) Ion trap mass spectrometric evidence for the metabolism of 3,4-(methylenedioxy)methamphetamine to the potent neurotoxins 2,4,5-trihydroxymethamphetamine and 2,4,5-trihydroxyamphetamine. Chem. Res. Toxicol. 4: 626–632

Logan, B. J., Laverty, R., Sanderson, W. D., Yee, Y. B. (1988) Differences between rats and mice in MDMA (methylenedioxymethylamphetamine) neurotoxicity. Eur. J. Pharmacol. 152: 227–234

Mennini, T., Garattini, S., Caccia, S. (1985) Anorectic effect of fenfluramine isomers and metabolites: relationship between brain levels and in vitro potencies on serotonergic mechanisms. Psychopharmacology 85: 111–114

Neff, N. H., Tozer, T. N. (1968) In vivo measurement of brain serotonin turnover. Adv. Pharmacol. 6A: 97–109

Nichols, D. E., Oberlender, R., Burris, K., Hoffman, A. J., Johnson, M. P. (1989) Studies of dioxole ring substituted 3,4-methylenedioxyamphetamine (MDA) analogues. Pharmacol. Biochem. Behav. 34: 571–576

Nichols, D. E., Brewster, W. K., Johnson, M. P., Oberlender, R., Riggs, R. M. (1990) Nonneurotoxic tetralin and indan analogues of 3,4-(methlenedioxy)amphetamine(MDA). J. Med. Chem. 33: 703–710

Paris, J. M., Cunningham, K. A. (1991) Lack of serotonin neurotoxicity after intraraphe microinjection of (+)-3,4-methylenedioxymethamphetamine (MDMA). Brain Res. Bull. 28: 115–119

Ram, F. S. F., Laverty, R. (1993) Differences in anorectic potency of fenfluramine, methylenedioxymethamphetamine (MDMA) and aminorex in rats and mice. Proc. Univ. Otago Med. Sch. 71: 29

Ricaurte, G., Bryan, G., Strauss, L., Seiden, L., Schuster, C. (1985) Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. Science 229: 986–988

Ricaurte, G. A., Forno, L. S., Wilson, M. A., DeLanney, L. E., Irwin, I., Molliver, M. E., Langston, J. W. (1988) (±) 3,4-Methylenedioxymethamphetamine selectively damages central serotonergic neurons in nonhuman primates. J. Am. Med. Assoc. 260: 51–55

Ricaurte, G. A., DeLanney, L. E., Irwin, I. Witkin, J. M., Katz, J. L., Langston, J. W. (1989) Evaluation of the neurotoxic potential of *N*,*N*-dimethylamphetamine: an illicit analog of methamphetamine. Brain Res. 490: 301–306

Roszkowski, A. P., Kelley, N. M. (1963) A rapid method for assessing drug inhibition of feeding behavior. J. Pharmacol. Exp. Ther. 140: 367–374

Rowland, N. E., Carlton, J. (1986) Neurobiology of an anorectic drug: fenfluramine. Prog. Neurobiol. 27: 13–62

Schmidt, C. J. (1987) Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. 240: 1–7

Schmidt, C. J., Kehne, J. H. (1990) Neurotoxicity of MDMA: neurochemical effects. Ann. NY Acad. Sci. 600: 665–680

Schmidt, C. J., Taylor, V. L. (1987) Depression of rat brain tryptophan hydroxylase following the acute administration of methylenedioxymethamphetamine. Biochem. Pharmacol. 36: 4095–4102

Schmidt, C. J., Wu, L., Lovenberg, W. (1986) Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue. Eur. J. Pharmacol. 124: 175–178

Schmidt, C. J., Levin, J. A., Lovenberg, W. (1987) In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. Biochem. Pharmacol. 36: 747–755

Schwartz, D., Hernandez, L., Hoebel, B. G. (1989) Fenfluramine administered systemically or locally increases extracellular serotonin in the lateral hypothalamus as measured by microdialysis. Brain Res. 482: 261–270

Shulgin, A. T., Sargent, T., Naranjo, C. (1969) Structure-activity relationships of one-ring psychotomimetics. Nature 221: 537–541

Steele, T. D., Nichols, D. E., Yim, G. K. W. (1987) Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [^{3}H]monoamines into synaptosomes from different regions of rat brain. Biochem. Pharmacol. 36: 2297–2303

Steranka, L. R., Sanders-Bush, E. (1979) Long-term effects of fenfluramine on central serotonergic mechanisms. Neuropharmacology 18: 895–903

Stone, D. M., Merchant, K. M., Hanson, G. R., Gibb, J. W. (1987) Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. Neuropharmacology 26: 1677–1683

Stone, D. M., Johnson, M., Hanson, G. R., Gibb, J. W. (1988) Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. 247: 79–87

Tozer, T. N., Neff, N. H., Brodie, B. B. (1966) Application of steady state kinetics to the synthesis rate and turnover time of serotonin in the brain of normal and reserpine-treated rats. J. Pharmacol. Exp. Ther. 153: 177–182

Wagner, G. C., Ricaurte, G. A., Seiden, L. S., Schuster, C. R., Miller, R. J., Westley, J. (1980) Long-lasting depletions of striatal dopamine and loss of dopamine uptake sites following repeated administration of methamphetamine. Brain Res. 181: 151–160

Yelnosky, J., Katz, R. (1963) Sympathomimetic actions of *cis*-2-amino-4-methyl-5-phenyl-2-oxazoline. J. Pharmacol. Exp. Ther. 141: 180–184

Young, R. (1992) Aminorex produces stimulus effects similar to amphetamine and unlike those of fenfluramine. Pharmacol. Biochem. Behav. 42: 175–178

Zheng, Y., Laverty, R. (1993) Neurotoxic effects of MDMA in different strains of mice. Proc. Univ. Otago Med. Sch. 71: 5–6

59. Schulz, J. B.; Henshaw, D. R.; Matthews, R. T.; Beal, M. F.: Coenzyme Q10 and nicotinamide and a-free radical spin trap protect against MPTP neurotoxicity. Exp. Neurol. 132:279–283; 1995.
60. Sentjurc, M.; Mason, R. P.: Inhibition of radical adduct reduction and reoxidation of the corresponding hydroxylamines in in vivo spin trapping of carbon tetrachloride-derived radicals. Free Rad. Biol. Med. 13:151–160; 1992.
61. Sloot, W. N.; Gramsbergen, J. B. P.: Detection of salicylate and its hydroxylated adducts 2,3- and 2,5-dihydroxybenzoic acids as possible indices for in vivo hydroxyl radical formation in combination with catechol- and indoleamines and their metabolites in cerebrospinal fluid and brain tissue. J. Neurosci. Methods 60:141–149; 1995.
62. Sprague, J. E.; Nichols, D. E.: The monoamine oxidase-B inhibitor L-deprenyl protects against 3,4-methylenedixoymethamphetamine-induced lipid peroxidation and long-term serotonergic deficits. J. Pharmacol. Exp. Ther. 273:667–673; 1995.
63. Stone, D. M.; Johnson, M.; Hanson, G. R.; Gibb, J. W.: Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. 247:79–87, 1988.
64. Stone, D. M.; Johnson, M.; Hanson, G. R.; Gibb, J. W.: Acute inactivation of tryptophan hydroxylase by amphetamine anlogs involves the oxidation of sulfhydryl sites. Eur. J. Pharmacol. 172:93–97; 1989.
65. Tanigawa, T.; Kotake, Y.; Reinke, L. A.: Spin trapping of superoxide radicals following stimulation of neutrophils with fMLP is temperature dependent. Free Rad. Biol. Med. 15:425–433; 1993.
66. Tabatabaie, T.; Floyd, R. A.: Susceptibility of glutathione peroxidase and glutathione reductase to oxidative damage and the protective effect of spin trapping agents. Arch. Biochem. Biophys. 314:112–119; 1994.
67. Wink, D. A.; Nims, R. W.; Saavedra, J. E.; Utermahlen, W. E. Jr.; Ford, P. C.: The Fenton oxidation mechanism: Reactivities of biologically relevant substrates with two oxidizing intermediates differ from those predicted for the hydroxyl radical. Proc. Natl. Acad. Sci. USA 91:6604–6608, 1994.
68. Yeh, S. Y.: Protection of 3,4-methylenedioxymethamphetamine (MDMA)-induced neurotoxicity by phenyl-t-butylnitrone (PBN), but not by salicylate (SALI), in rats. Soc. Neurosci. Abstr. 25:973; 1995.
69. Yeh, S. Y.: *N*-tert-butyl-α-phenylnitrone (PBN) protects against 3,4-methylenedioxymethamphetamine (MDMA)-induced neurotoxicity in rats. Submitted for review.
70. Yeh, S. Y.; Hsu, F. L.: The neurochemical and stimulatory effects of putative metabolites of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in rats. Pharmacol. Biochem. Behav. 39:787–790; 1991.
71. Yousif, M. Y.; Fitzgerald, R. L.; Narasimhachari, N.; Rosecrans, J. A.; Blanke, R. V.; Glennon, R. C.: Identification of metabolites of 3,4-methylenedioxymethamphetamine in rats. Drug Alcohol Depend. 26:127–135; 1990.
72. Zhao, Z.; Castignoli, N.; Ricaurte, G. A.; Steele, T.; Martello, M.: Synthesis and neurotoxicological evaluation of putative metabolites of the serotonergic neurotoxin 2-(methylamino)-1-[3,4-[methylenedioxy)phenyl]propane[(methylenedioxy)methamphetamine. Chem. Res. Toxicol. 5:89–92; 1992.