Ecstasy during Loud Noise Exposure Induces Dramatic Ultrastructural Changes in the Heart

Marco Gesi1), Paola Soldani1), Paola Lenzi1), Michela Ferrucci1), Alberto Giusiani2), Francesco Fornai1) and Antonio Paparelli1)

1)Department of Human Morphology and Applied Biology and 2)Department of Public Health, University of Pisa, Italy
(Received October 24, 2001; Accepted January 28, 2002)

Abstract: The acute toxicity induced by 3,4-methylenedioxymethamphetamine appears as rhabdomyolysis involving the myocardium (myocytolysis) and it is often suspected to be responsible for sudden death. In line with this, cardiac symptoms such as tachycardia, hypertension, and arrhythmia are present in persons abusing ecstasy. In most cases, ecstasy is abused in loud noise, which in itself might affect the myocardium. To our knowledge no study has investigated the concomitant exposure to ecstasy and loud noise in order to evaluate the role of the loud noise in modulating MDMA toxicity. In the present study, we analyzed whether cardiac effects following a typical “binging” pattern of MDMA administration are enhanced by concomitant exposure to loud noise. Our findings did not show any myocardial lesion detectable under light microscopy. In contrast, alterations were visible at the ultrastructural level as mitochondrial changes. In particular, we found a marked enhancement in the number of altered mitochondria when MDMA was administered during exposure to loud noise.

3,4-M ethylenedioxymethamphetamine (MDMA, ecstasy) is an amphetamine derivative which has become increasingly popular as a substance of abuse among young people. In recent years, toxic effects induced by MDMA have been reported both in animal models (Ricaurte et al. 2000) and in man (Dowling 1986; McCann et al. 2000). In particular, MDMA self-administration in man leads to early severe toxicity involving the myocardium (Milroy et al. 1996). These effects are believed to contribute to dramatic and unpredictable events up to sudden death after intake of MDMA.

There is an increasing number of reports showing delayed neuropsychiatric deficits in chronic MDMA abusers. While a great number of animal studies have analyzed the mechanisms responsible for MDMA-induced neurotoxicity, with an emphasis on the toxicity produced by this compound on cerebral monoaminergic systems (Battaglia et al. 1987 & 1988; Commins et al. 1987), it is surprising that analogous experimental studies on effects of MDMA on the cardiovascular system were not carried out. This is unexpected, even considering the frequency and the severity of early cardiovascular impairment following intake of MDMA in man (Henry et al. 1992).

Considering this point, we wanted to evaluate the early events and the preferential targets within the myocardium at subcellular levels. On the other hand, since we want to reproduce the natural pattern of recreational MDMA intake, we have administered the drug as multiple doses at constant time intervals. Moreover, in most cases, this substance is abused in the presence of loud noise, which by itself is known to affect the myocardium (Soldani et al. 1997).

In the present study we have examined the consequence of repeated MDMA administration either alone or during exposure to noise. We selected low doses of the drug in order to evaluate the finest ultrastructural changes occurring in the mouse myocardium. In these experimental conditions it was also possible to determine whether a high environmental noise level could enhance the myocardial effects produced by low doses of ecstasy.

The pattern of stimulation (either loud noise or MDMA administration) was chosen to imitate as close as possible what generically occurs during recreational abuse: constant noise for 6 hr, associated with a binging dosage of MDMA. We used mice since they exhibit a greater similarity with man compared with rats (Steinfath et al. 1992; Fornai et al. 2001).

Materials and Methods

Animals. Male C57 Black mice (C57BL/6) 9–10 weeks old obtained from Harlan Industries (San Pietro al Natisone, Italy) were used in these experiments. They were housed under controlled conditions (12 hr light/dark cycle with lights on between 7 a.m. and 7 p.m., 60% humidity) and were fed and allowed to drink water ad libitum.

The toxicity of amphetamine derivatives critically depends on room temperature (Albers & Sonsalla 1995) and the number of ani-
mals per cage (Fornai et al. 2001), we carried out the experimental schedule at constant room temperature (21 °C).


Noise exposure. Noise was produced by two loudspeakers (15 W), driven by a white-noise generator (0–26 kHz), and installed 40 cm apart on opposite sides of the cage. The noise level was set at 100 dBA uniformly throughout the cage, as monitored by a sound level meter (Quest Electronics 215). This noise level was selected based on the intensity occurring in discotheques where the M D M A is often used.

Each treated animal was exposed for 6 hr. To avoid the influence of cage-stress on evaluation of effects due to noise exposure, control mice were kept in the above described cage during the corresponding period of time, without noise stimulation.

Drug treatment. Ecstasy was purchased by the Department of Public Health, University of Pisa. Authentic M D M A hydrochloride was obtained from solid samples as described by Dal Cason (1989), modified by repeating the crystallization procedure twice. The purity of chloride-containing crystals was verified by measuring the melting point and by running gas chromatography (G.L.C) coupled with mass-spectrometry before the experiments.

In pilot studies we tried various dosages of M D M A in order to select the highest dose, which by itself did not produce any morphological alteration in the myocardium. This dose however, produced a frank neurotoxicity. Therefore, M D M A was administered intraperitoneally at the dose of 20 mg/kg, with an interval of 2 hr. In animals which received combined treatment (noise + M D M A), the first injection of M D M A occurred at the beginning of noise exposure. The following M D M A injections were carried out at 2 hr intervals, during noise exposure (2nd and 3rd injection). Control and noise exposed mice received saline solution intraperitoneally at the same time used for M D M A-treated mice.

Experimental procedure. At the end of the treatment, mice were anaesthetised with ether and perfused through the left ventricle with the fixing solution (1.25% glutaraldehyde in 0.08 M cacodylate buffer, 0.03 M CaCl2, pH 7), in order to obtain an optimal preservation of the heart. The fixing solution for 1 hr. Specimens were then post-fixed for 2 hr at 4 °C in 1% buffered OsO4, dehydrated in ethanol, and embedded in Epon-araldite. Sections were observed under a Jeol JEM 100 SX transmission electron microscope.

Since we have previously found that effects of loud noise on the myocardium are mediated by an increase of noradrenergic activity (Breschi et al. 1994), we dissected the specific myocardium from the right atrium and ventricle, which receives the most abundant noradrenergic innervation.

Statistical analysis. From each mouse, we chose two tissue blocks at random and we took 10 electron micrographs, which were examined at a final magnification of ×10,000. The extent of the damage was measured counting the number of altered mitochondria out of the total number, and expressed as percentage values.

Results were calculated as means ± S. E. M. for each group. Comparisons between groups were carried out using one-way analysis of variance (A N O V A) with Sheffe’s post-hoc analysis. The null hypothesis was rejected when P < 0.05.

Table 1. Percentage of altered mitochondria after ecstasy and loud noise.

<table>
<thead>
<tr>
<th></th>
<th>Atrium</th>
<th>Ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.50 ± 1.07</td>
<td>3.65 ± 0.69</td>
</tr>
<tr>
<td>Loud noise</td>
<td>15.37 ± 3.22</td>
<td>10.5 ± 3.27</td>
</tr>
<tr>
<td>M D M A</td>
<td>11.68 ± 1.49</td>
<td>9.41 ± 3.39</td>
</tr>
<tr>
<td>M D M A + loud noise</td>
<td>28.89* ± 11.71</td>
<td>16.79* ± 3.75</td>
</tr>
</tbody>
</table>

The extent of the damage was measured by the percentage of altered mitochondria. Data refer to the percentage of altered mitochondria counted in micrographs selected randomly at a final magnification of ×10,000. For each group a total of 100 counts were performed from three repeated experiments. Values are given as the means of 10 mice per group. Comparison between groups was carried out using Analysis of Variance with Sheffe’s post-hoc analysis.

* P < 0.05 compared with controls.
31 NOISE ENHANCES ECSTASY TOXICITY

Fig. 2a. Ventricular myocyte from control mouse. Normal mitochondria, with dense matrix, are intercalated among myofibrils. m, mitochondria; f, myofibrils; l, lipidic droplet. Magnification ×17,500. 2b. Ventricular myocyte from noise-exposed mouse. Only a few mitochondria are altered (arrows). m, mitochondria; f, myofibrils. Magnification ×17,500. 2c. Ventricular myocyte from MDMA treated mouse. A few mitochondria show alterations (arrows). m, mitochondria; f, myofibrils. Magnification ×17,500. 2d. Ventricular myocyte from mouse exposed to MDMA and noise. Despite myofibrils exhibit a normal ultrastructure, mitochondria appear dramatically damaged. In particular, note disarrangement of cristae and dilution of the matrix (arrows). m, mitochondria; f, myofibrils; l, lipidic droplet. Magnification ×17,500.

Several side-effects appear after ecstasy intake (Green et al. 1995; Burgess et al. 2000). Apart from chronic neurotoxic alterations, which have been widely investigated (McGuire & Fahy 1991; Ricaurte et al. 1992), early toxic effects mainly involving the myocardium might be dramatic up to sudden death (Henry et al. 1992; McCann et al. 2000). Cardiac effects, when including minor changes, occur frequently after intake of MDMA. In line with this, persons abusing ecstasy typically suffer cardiac symptoms, such as tachycardia, hypertension, and arrhythmia (Miller et al. 1996). Nonetheless, the mechanisms by which MDMA-induced early toxicity occur, are not investigated and deserve further experimental studies.

The aim of this study consisted in determining at a morphological level the nature and the extent of the myocardial damage after MDMA administration. Moreover, since abuse of ecstasy in man is often associated with loud noise in overcrowded areas, we tried to reproduce these en-
virential conditions by exposing M D M A-treated and non-treated mice to continuous, loud noise exposure.

Postmortem reports after fatal ecstasy intake describe rhabdomyolysis, often involving the myocardium (myocytolysis) (Screaton et al. 1992). Despite these severe findings at early time intervals after lethal M D M A intoxication, there was no histological alteration in the myocardium in mice receiving low doses of M D M A. The same was observed after exposure to noise. This might depend on the low dose administered, which may be not sufficient to provoke gross changes, detectable at histological level. Indeed, in pilot studies we found that higher doses of M D M A produced by itself morphological alterations in the myocardium. These dosages were not included in the present study since we want to investigate at a later time whether loud noise exposure changes the threshold for cardiotoxicity. Nonetheless, in future experiments it would be worthwhile to investigate the combined effects of loud noise with doses of M D M A which by themselves produce myocardial alterations.

The myocardial alterations observed here were visible only at the ultrastructural level and there were slight increases in the number of altered mitochondria which was constantly present in both the atrium and the ventricle of M D M A-exposed mice.

Remarkably, when M D M A was administered during noise exposure, a dramatic and significant increase in the number of altered mitochondria was measured both in atria and in ventricles. These ultrastructural alterations occurred in the absence of any detectable change when the myocardium was observed at histological level.

These cardiac effects are likely to be sustained by increased noradrenaline release and subsequent mitochondrial calcium entry as indicated by previous studies carried out on both M D M A (Steel et al. 1989) and noise exposure (Gesi et al. 2000). Therefore, synergism between the two stimuli occurring at morphological level might be explained by an overlapping of effects when observed at a biochemical level.

While it is well documented that M D M A toxicity critically depends on room temperature and self-aggregation (Ricaurte et al. 2000), there are no data about the influence of loud noise on the effects produced by M D M A. This is even more crucial considering that M D M A self-administration occurs during loud noise exposure (high intensity for several hours).

In the present study we have been able to detect in an animal model fine ultrastructural changes in the myocardium occurring early after exposure to ecstasy and loud noise, which are dramatically enhanced by loud noise.

References


Soldani, P., A. Pellegrini, M. Gesi, P. Lenzi, R. Cristofani & A. Paparelli: SEM/TEM investigation of rat cardiac subcellular...
alterations induced by changing duration of noise stress. Anat.
alters biogenic amines and metabolites in mouse brain and heart.
Steinfath, M., Y. Y. Chen, J. Lavicky, O. Magnussen, M. Nose, S.
Rossaw, W. Schmitz & H. Scholz: Cardiac α₁-adrenoceptor den-
sities in different mammalian species. Brit. J. Pharmacol. 1992,
107, 185–188.
Tomanek, R. J. & U. L. Karlsson: Myocardial ultrastructure of