Int J Legal Med (2003) 117 : 2–9
DOI 10.1007/s00414-002-0290-2

ORIGINAL ARTICLE

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Immunohistochemical demonstration of the amphetamine derivatives 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) in human post-mortem brain tissues and the pituitary gland

Received: 1 October 2001 / Accepted: 20 December 2001 / Published online: 25 January 2003

Abstract Abuse of amphetamine derivatives such as 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) is an important issue in current forensic practice and fatalities are not infrequent. Therefore, we investigated an immunohistochemical method to detect the amphetamine analogues MDMA and MDA in human tissues. For the staining procedure, the Catalysed Signal Amplification (CSA) method using peroxidase (HRP) provided by Dako® and specific monoclonal antibodies were used. Appropriate controls for validation of the technique were included. The distribution of these designer drugs was studied in various brain regions including the four lobes, the basal ganglia, hypothalamus, hippocampus, corpus callosum, medulla oblongata, pons, cerebellar vermis and, additionally, in the pituitary gland. A distinct positive reaction was observed in all cortical brain regions and the neurons of the basal ganglia, the hypothalamus, the hippocampus and the cerebellar vermis but in the brainstem, relatively weak staining of neurons was seen. The reaction presented as a mainly diffuse cytoplasmic staining of the perikaryon of the neurons, and often axons and dendrites were also visualised. In addition, the immunoreactivity was present in the white matter. In the pituitary gland, however, distinct immunopositive cells were observed, with a prominent heterogeneity. The immunohistochemical findings were supported by the toxicological data. This immunostaining technique can be used as evidence of intake or even poisoning with MDMA and/or MDA and can be an interesting tool in forensic practice when the usual samples for toxicological analysis are not available. Furthermore, this method can be used to investigate the distribution of these substances in the human body.

Keywords 3,4-Methylenedioxymethamphetamine (MDMA) · 3,4-Methylenedioxyamphetamine (MDA) · Immunohistochemistry · Human brain · Pituitary gland

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Introduction

Abuse of amphetamine and its derivatives is an important problem in current forensic practice. Moreover fatal and nearly fatal cases of intoxication are not infrequent [1]. The post-mortem distribution has barely been explored for amphetamine analogues, except for some case reports [2, 3, 4, 5, 6] and animal experiments considering this aspect for amphetamine or its analogues are scarce [7, 8, 9].

Immunological methods are routinely used for detection of illegal drugs in clinical and forensic toxicology, mainly in urine screening tests. However, the principle of antigen-antibody recognition can also be applied in histological specimens (immunohistochemistry), allowing detection of drugs in tissue sections. Immunofluorescence procedures have previously been applied successfully in animal experiments e.g. detection of morphine in rat tissues [10], demonstration of tetrahydrocannabinol [11] and phenobarbital [12] in mice tissues. Immunohistochemical demonstration of morphine and methadone in brain sections of overdose victims has recently been published [13, 14] and insulin was demonstrated at injection sites by means of immunohistochemistry [15, 16, 17].

Part of the behavioural, psychotomimetic and neurochemical effects of MDMA (e.g. increase of body temperature, mood alterations, anxiolytic-like effects) may be explained by the effect on the serotonergic system [18, 19]. Interaction of MDMA with post-synaptic as well as

pre-synaptic 5-hydroxytryptamine (5-HT, serotonin) recognition sites is postulated [18]. In animal studies, high affinity of MDMA for 5-HT_2 and 5-HT_{1A} serotonin receptors [18] as well as for 5-HT uptake sites was proven [20]. The neurotoxicity of MDMA on 5-HT neurons was also investigated in humans and subtle, but significant cognitive deficits were noticed [21].

At present, immunohistochemical methods are used to investigate the biological effects of MDMA, for example, to demonstrate reductions in 5-HT axon density in rats and monkeys [22] or rhabdomyolysis [4, 23]. Ishiyama et al. were able to demonstrate methamphetamine in mice tissues [24]. To the best of our knowledge, the amphetamine derivatives 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") have not yet been demonstrated in human tissues.

In this study, we applied the CSA immunostaining method—known to be the most sensitive method at present—for the microscopical immunodetection of MDMA and MDA. In particular, the distribution of MDMA and MDA in slides of the human brain and the pituitary gland of two fatal intoxications was investigated and correlated with the toxicological findings.

Case histories

Case 1

A 23-year-old man was found dead, sitting on a chair in a bar. Upon examination, 28 h after death, the body was 186 cm in length and weighed about 100 kg. No conspicuous lesions or injection sites were noticed. At autopsy, both lungs weighed 1,620 g and severe pulmonary congestion and oedema, numerous Tardieu spots on the pleurae and the pericardium were noticed. The heart weighed 405 g and a persistence of the left vena cava superior was found. The brain weighed 1,545 g and congestion and oedema were confirmed by histological examination. The macroscopical and microscopical findings were consistent with an acute to subacute cardiopulmonary failure as the mechanism of death.

MDMA and MDA concentrations were determined in femoral blood and brain using a HPLC (high pressure liquid chromatography) procedure with fluorescence detection [5, 25]. For additional confirmation, a fully quantitative LC-MS (liquid chromatography-mass spectrometry) assay was used for the determination of all specimens. Cocaine, benzoylecgonine, cocaethylene, ecgonine methylester, amphetamine, MDMA, MDEA (3,4-methylenedioxyethylamphetamine) and MDA were detected using chromatographic analysis: HPLC-DAD (HPLC with diode array detection), LC-MS, GC-NPD (gas chromatography with nitrogen-phosphorus detection) and GC-MS of the urine sample [26, 27]. The blood alcohol level was quantified by head-space GC analysis.

Case 2

The 23-year-old man was found dead at home, naked, and his clothes were found near the body (as if he had just undressed). The body was in a state of beginning putrefaction and the cadaveric signs combined with the police inquiry revealed that the post-mortem interval was about 3 days. The body weighed 56 kg and was 175 cm in length. At autopsy, both lungs weighed 1,410 g and showed obvious congestion and oedema. Apart from congestion and moderate putrefaction, the other organs showed no conspicuous anomalies. The heart and brain weighed 315 and 1,405 g, respectively. Microscopical examination confirmed the general congestion and revealed signs of shock (such as leucocyte sludging, micro-embolisms), mainly in the sections of the heart, lungs, liver, kidneys and brain. Eosinophilic cylinders were found in the renal tubuli, but myoglobin staining was negative. In several brain regions, apart from congestion and oedema, small—mainly perivascular—bleedings were found. The medico-legal findings were consistent with a disseminated intravascular coagulation (DIC) induced by hyperthermia.

Amphetamines and related compounds were screened by routine methods such as GC-MS and HPLC-DAD, while quantitative results were obtained by GC-MS and LC-MS.

Materials and methods

For both fatalities, since drug abuse was suspected, the standard protocol used at our department was applied and appropriate sampling for toxicological and histological examination was performed. Small parts of the four brain lobes were sampled for drug assay and for each lobe, cortex and medulla were isolated. Thereafter, the brain was fixed in 4% buffered formaldehyde for 3–4 weeks. On dissection, samples of various brain regions were taken, followed by embedding in paraffin. Tissue sections were prepared from the frontal, temporal, parietal and occipital regions, the medulla oblongata, pons, cerebellar vermis, corpus callosum, hippocampus, the basal ganglia (mammillary bodies, lentiform nucleus, caudate nucleus and thalamus), the adjacent hypothalamus, and the pituitary gland.

All similar samples in the control case 00/116 were taken as blanks for drug assay and negative control tissue for immunohistochemistry: a 28-year-old woman [post-mortem interval (PMI) of 48 h] was murdered by a shotgun through the heart and lungs. In a few staining procedures, at random samples of five other control cases were used. These were a 32-year-old female, murdered by thoracic gunshot wounds (case number 00/8; PMI of 36 h), a 27-year-old female, murdered by multiple stab wounds (case number 00/14; PMI of 56 h), a 26-year-old man, murdered by a gunshot through the heart (case number 00/19; PMI of 42 h), a 17-year-old man, who died due to polytrauma after a traffic accident (case number 01/68, PMI of 91 h), and a 30-year-old female, murdered by multiple stab wounds in thorax and neck (case number 01/181; PMI of 58 h). In all control cases, extensive toxicological investigations were negative.

Antibodies and immunostaining procedure

Monoclonal antibodies which specifically recognise MDMA and MDA were kindly supplied by Microgenics Corp. (Fremont, Calif.): they were purified from mouse ascites and available in two clones (clones 1A9 and 5C2).

For the staining procedure, the Dako® Catalysed Signal Amplification (CSA) system, peroxidase (HRP) was used (supplied as a

kit; Code K 1500). CSA is a very sensitive immunohistochemical staining procedure incorporating a signal amplification method based on the peroxidase catalysed deposition of a biotinylated phenolic compound, followed by a secondary reaction with streptavidin-peroxidase (User instructions, Dako® Catalysed Signal Amplification System). Sections were deparaffinised and rehydrated according to standard protocols. After blocking endogenous biotin and suppressing non-specific background staining as prescribed in the procedure, the primary monoclonal mouse antibody was applied to the tissues and an incubation of 15 min followed. For the primary antibodies, dilution series ranging from 1:20 to 1:5,000 were tested and the best results were obtained with dilutions between 1:150 and 1:500. The slides were then gently rinsed with TBST buffer solution (Tris buffered saline with Tween 20) and placed in a fresh TBST buffer bath 3 times for 3–5 min each. The procedure was then continued by sequential 15 min incubations with biotinylated link antibody, streptavidin-biotin complex, amplification reagent and streptavidin-peroxidase, respectively. The staining was completed by a 3–5 min incubation with 3,3'-diaminobenzidine tetrahydrochloride (DAB) which is oxidised to a brown-coloured precipitate by HRP. Hematoxylin counterstaining was sometimes performed (e.g. in the pituitary gland in order to visualise the basophilic cells).

For the pituitary gland, a Periodic Acid Schiff (PAS)-orange G-staining on an immediately adjacent slide was performed in order to discern the cell subtypes of the pituitary gland (acidophils, basophils and chromophobes).

In all staining experiments of the two amphetamine fatalities, a simultaneous incubation was performed with analogue sections from control cases.

A positive control staining (recommended in the CSA kit) with monoclonal mouse antibody to human B-cell ($CD23_1$) on formalin-fixed and paraffin-embedded lymph node (from a patient with malignant B-cell lymphoma) and palatine tonsil tissue was performed. At the same time, as an additional negative control, the staining procedure with the IgG_1 fraction from normal mouse (provided in the kit) and with the antibodies recognising MDMA and MDA on adjacent sections of the lymph node and palatine tonsil, was applied.

In order to test the specificity of the antibodies, we checked whether it is possible to saturate the antibody binding sites with its specific antigens and thus induce a negative immunodetection. Therefore, either MDMA or MDA were added at various concentrations (dilution solution series from 10^{-12} g/ml to 1.5×10^{-3} g/ml) to the antibody solution. This solution was placed on a rotary mixing device during 24 h (at 4°C) prior to incubation of the slides. The same procedure was performed using PMA (*para* -methoxyamphetamine) or AMP (amphetamine). For negative control incubations, the antibody was replaced by IgG_1 fraction from normal mouse serum (supplied with the CSA kit) for each brain region. A few random negative controls were performed in which phosphate buffered saline (PBS) or bidistilled water (used to make the MDMA or MDA solutions) were used instead of the primary antibody.

To exclude that MDMA or MDA added to the antibody solution and acting as a salt would prevent antigen-antibody binding, an excess of MDMA or MDA was added to antibody solutions in another immunodetection protocol. This concerned the immunodetection of two peroxisomal enzymes [catalase (CAT) and alanine-glyoxylate aminotransferase (AGT)] in liver slides of cases and controls [28].

Results

Immunohistochemistry

An overview of the immunohistochemical results is presented in Table 1. Positive immunoreactions were obtained in the neurons of all brain regions of both fatalities, except for the corpus callosum due to the absence of neuronal cell bodies at that particular site. A distinct positive reaction was seen in all cortical regions of the brain lobes, the neurons of the basal ganglia, and the cerebellar vermis. Relatively weak staining of the neurons was found in the brainstem. In addition, distinct reactivity was also observed at the level of the white matter fibres in the slides of the basal ganglia, the brainstem and corpus callosum in both cases. A rather weak immunoreactivity of the white matter in the cerebral lobes was noticed which is a discrepancy with the results from chromatographic analysis in the homogenates (see toxicological data).

Table 1 Immunoreactions in neurons of various brain regions in fatalities and control cases

Brain region	Case 1 (00/112)	Case 2 (01/34)	Control cases
	Neurons		
Frontal lobe	+	+	–
Parietal lobe	+	+	–
Temporal lobe	+	+	–
Occipital lobe	+	+	–
Caudate nucleus	+	+	–
Thalamus	+	+	–
Lentiform nucleus	+	+	–
Medulla oblongata	±	±	–
Pons	±	±	–
Vermis cerebelli	+	+	–
Mammillary bodies	+	+	–
Hypothalamus	+	+	–
Hippocampus	+	+	–
Corpus callosum	N.A.	N.A.	N.A.

+ distinct immunoreactivity
± weak immunoreactivity
– no immunoreactivity
N.A. not applicable

Figures 1a, 1d, 2b and 3b show immunoreactive neurons in the parietal and frontal region, the cerebellar vermis and the hippocampus, respectively. The reaction presented as a mainly diffuse brownish cytoplasmic staining of the perikaryon of the neurons. When the orientation of the section plane was appropriate, staining of the axons and dendrites was noticed. In particular, this was obvious in the Purkinje cells presented in Fig. 2b where the course of the dendrites to the molecular layer could be followed. In addition, the granular layer cells of the cerebellar vermis were positive, but at higher magnification, a heterogeneity in the staining pattern of these cells was observed (see Fig. 2b). The cells of the dentate gyrus in the hippocampus were also visualised (see Fig. 3c), however, the heterogeneity was less pronounced. When comparing all brain regions, differences in staining intensity and number of positive neurons were seen, but these were not quantified.

In Fig. 4, a macroscopical overview of the immunoreactivity in the sections of the cerebellar vermis in Case 1 and in a control case is presented. The focal staining pattern in the control differed from that in the case, as the white matter was unreactive in the control and on micro-

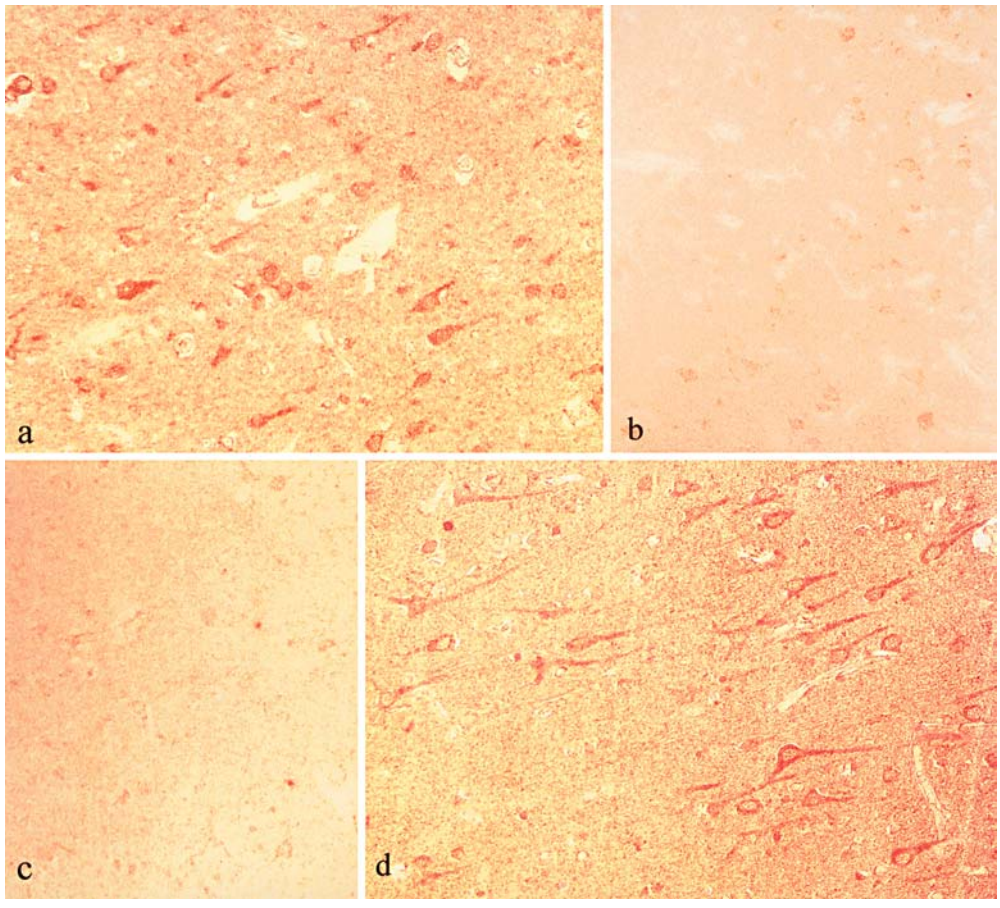

Fig. 1 **a** Immunohistochemical staining of neurons (nerve cell bodies, axons and dendrites) in the parietal lobe of case 1. **b** Staining of the parietal lobe of case 1 after saturation of the antibody solution with MDMA, and as a result, negative immunodetection was induced. **c** Negative immunohistochemical staining of the parietal cortex in the control case. (antibody clone 1A9; magnification **a** 190×, **b** 140× and **c** 140× respectively). **d** Immunohistochemical staining of neurons (nerve cell bodies, axons and dendrites) in the frontal lobe of case 1 (antibody clone 5C2, magnification 190×)

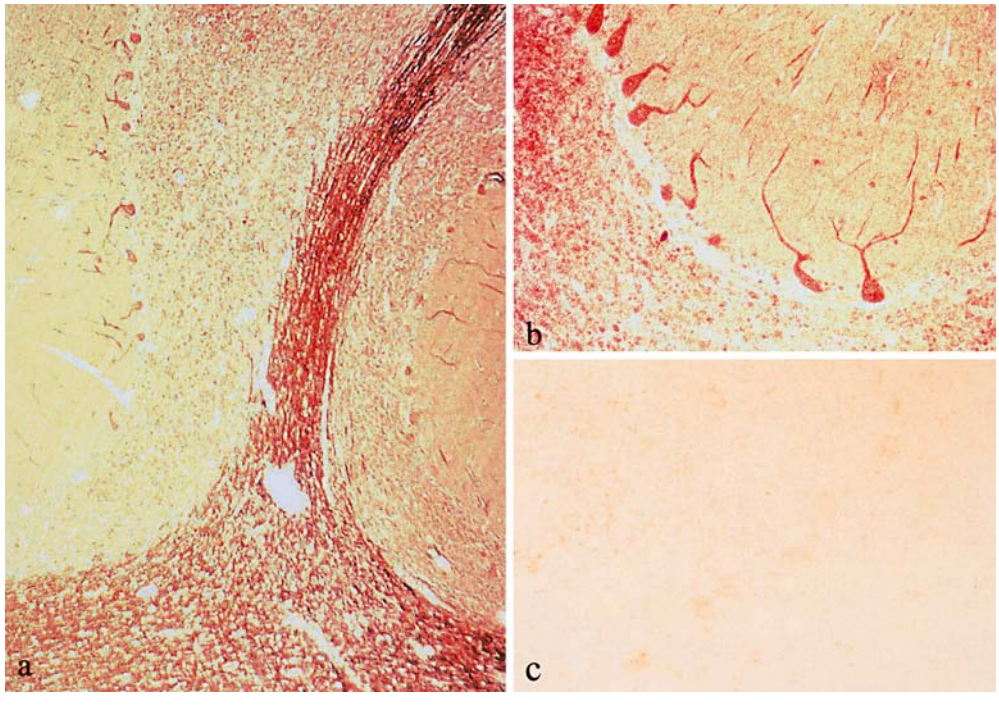

Fig. 2 **a** Positive immunostaining of the cerebellar vermis of case 2: the nerve cell bodies, axons and dendrites of the Purkinje cells are clearly visible. The granular layer cells can also obviously be discerned. In addition, staining at the level of the white matter fibres is seen (overview magnification 95×). **b** Detail of the immunoreaction in the cerebellar vermis of case 2 (magnification 270×). **c** Negative immunohistochemical staining of the cerebellar vermis in a control case. (*For all pictures: staining using antibody clone 1A9*)

scopical examination, no immunoreactive neurons were seen in the control.

Positive staining reactions were found for both antibody clones; however, the staining aspect of both clones was somewhat different. The two neuronal staining patterns are presented in the parietal and frontal lobe (Fig. 1a and d, respectively). When antibodies of the 5C2 clone were used, the staining in the perikaryon was usually somewhat

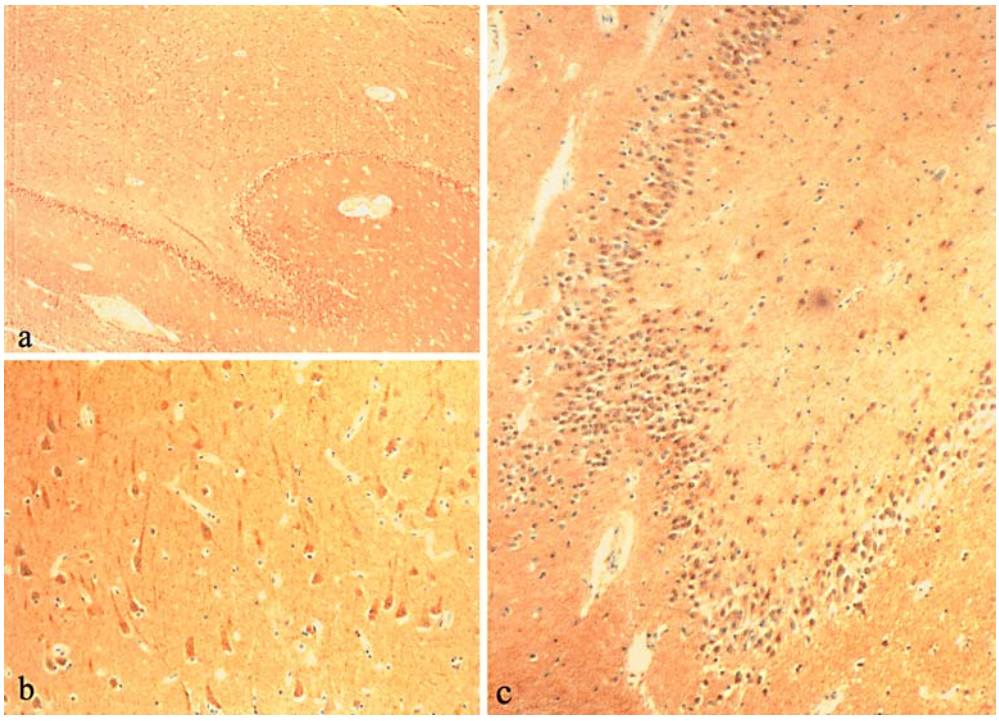

Fig. 3 Staining of neurons in the hippocampus of case 2 (antibody clone 1A9). **a** Overview (magnification 25×). **b** Detail of the cortical neurons (magnification 100×). **c** Detail of the cells of the dentate gyrus (magnification 100×)

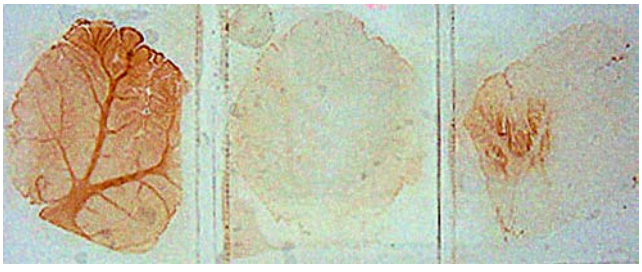

Fig. 4 Macroscopical overview of the slides of the cerebellar vermis (staining using antibody clone 1A9): *left* positive immunostaining in case 1, *middle* immunostaining in case 1 after saturation of the antibody binding sites with MDMA inducing a negative result, *right* negative immunohistochemical staining of the cerebellar vermis in a control case (00/116)

flocky. Incubation with 1A9 rendered a more diffuse colouring in the neuronal cytoplasm and the DAB precipitate was more heterogenous. Variations in staining intensity were also noticed when both clones were compared. Although both clones distinctly revealed visualisation of the cortical neurons, we further proceeded with 1A9 because this antibody clone produced a crisp microscopical image.

The validation procedure for positive controls (as recommended in the kit), using anti-$CD23_1$ on formalin-fixed and paraffin-embedded lymph node and palatine tonsil tissue, was applied and found to be positive. The same procedure with IgG_1 fraction and with the antibodies recognising MDMA and MDA on lymph node and tonsil sections was negative.

In the control cases, no neurons were revealed, except for a very faint diffuse cytoplasmic staining in the perikarya (see Fig. 1c and Fig. 2c, parietal cortex and cerebellar vermis, respectively).

Addition of pure MDMA or MDA to the antibody solution in the high concentration of the series, totally abolished the staining reaction or reduced staining to the background level as seen in the control cases (see Fig. 1b and Fig. 4, parietal lobe and cerebellar vermis, respectively). In the lower MDMA concentration range, a gradient in the staining intensity was observed. These results point to the specificity of the antibodies. Addition of PMA or amphetamine to the antibody solution was not able to induce negative immunoreactivity. In addition, incubation with mouse IgG_1 fraction was negative in the case and control tissues (data not shown).

The immunodetection of catalase and alanin/glyoxylate aminotransferase in the case and control liver was not affected by addition of a similar amount of MDMA used to saturate the MDMA antibody.

Figure 5 shows the results in the pituitary gland. Distinct immunoreactivity was observed in many cells, together with a prominent heterogeneity. As a result, different types of staining intensity can be discerned: highly intensely staining cells and abundant positive cells but also some weakly stained and a few negative cells could be noticed. Comparison of these results with the PAS-orange G-staining in adjacent sections indicates that the strongly stained cells correspond to the acidophils. The weakly stained and negative cells are assumed to be the basophils and chromophobes, respectively. The cells of the latter subtype are indeed a minority.

Toxicological data

In Table 2, the MDMA, MDA, PMA and amphetamine concentrations in femoral blood and homogenates of the

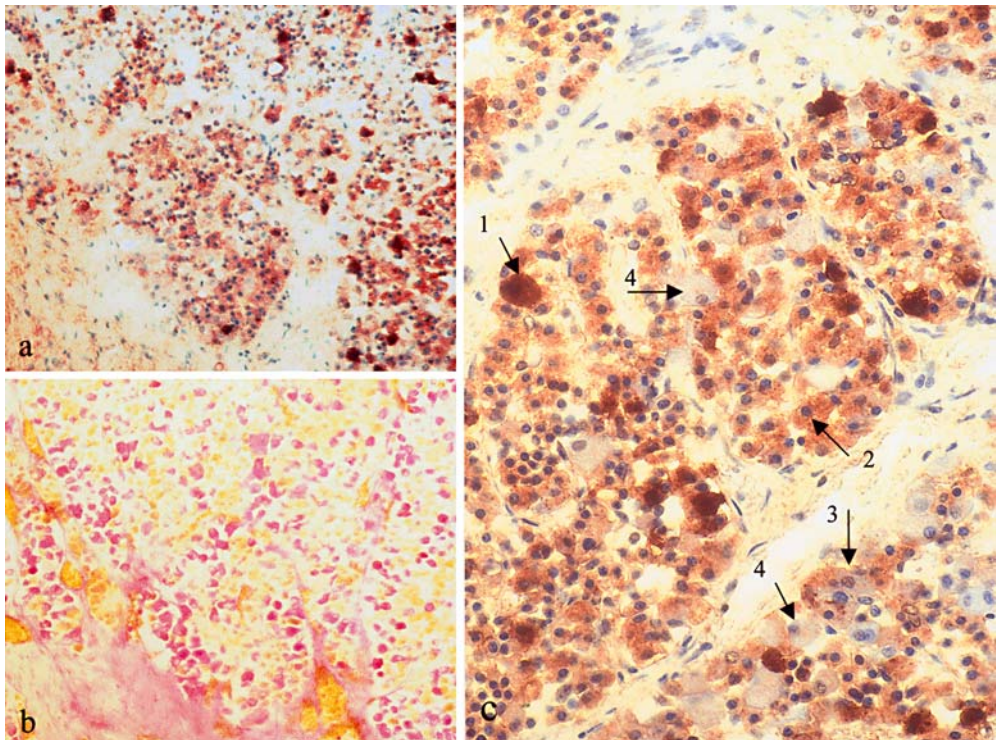

Fig. 5 **a** Overview of MDMA immunoreactivity in the pituitary gland (antibody clone 1A9; magnification 140×). **b** PAS Orange-G staining of the immediately adjacent slide visualising clearly the acidophilic (*orange colour*) and basophilic cells (*violet colour*), (magnification 140×). **c** Detail of the staining reaction for MDMA in the pituitary gland (antibody clone 1A9; magnification 300×). Following types of staining can be discerned (*see arrows*): *1*, *2* obviously positive cells showing variable staining intensity (heterogeneity); the highly intensively stained cells obscure the nucleus (see arrow *1*), *3* cells having weak immunoreaction, *4* negative cells

Table 2 Toxicological data in femoral blood, cortex and medulla of the four brain lobes in the two fatalities

Samples tested	Case 1 (00/112)		Case 2 (01/34)			
	MDMA	MDA	MDMA	MDA	PMA	AMP
Femoral blood (µg/ml)	3.07	0.09	1.22	0.39	1.43	0.22
Brain region (µg/g)						
Frontal lobe						
Cortex	13.65	0.15	1.66	0.57	3.89	0.33
White matter	13.05	0.13	1.89	0.68	3.52	0.49
Temporal lobe						
Cortex	15.42	0.16	1.62	0.81	3,25	0.43
White matter	13.53	0.21	3.00	0.81	4.40	0.44
Parietal lobe						
Cortex	15.19	0.23	2.61	0.57	3.92	0.33
White matter	12.46	0.15	3.05	0.62	2.94	0.43
Occipital lobe						
Cortex	15.98	0.18	1.87	0.52	3.23	0.33
White matter	10.87	0.19	3.30	0.57	4.74	0.32
Brainstem	13.18	0.22	1.95	0.76	3.20	0.35
Cerebellum	11.69	0.23	0.98	0.49	2.37	0.66

MDMA 3,4-methylenedioxymethamphetamine
MDA 3,4-methylenedioxyamphetamine
PMA para-methoxyamphetamine
AMP amphetamine

cortex and white matter of the four brain lobes are presented. The analysis revealed also an important level of the drugs in the white matter.

In the first fatality, the routine analysis of blood and urine disclosed the presence of a high level of amphetamines (68.4 µg/ml in urine, immunoassay result) and trace amounts of benzoylecgonine (0.7 mg/ml in urine, chromatographic result). Head-space GC analysis demonstrated the absence of ethanol in blood and urine. In addition, the presence of MDMA in blood was demonstrated by HPLC-DAD and GC-MS.

For the second case, apart from the data presented in Table 2, no alcohol or other drugs were found.

Discussion

In this study, a method for immunohistochemical detection of MDMA and MDA in human tissues is presented. In addition, we examined the distribution of MDMA and MDA in various brain regions and the pituitary gland.

Positive immunostaining for MDMA and MDA was established in the neuron cell bodies, axons and dendrites in various regions of the brain in two cases of fatal intoxication. However, the immunoreactivity was also present at the level of the white matter fibres. The toxicological data confirmed that substantial concentrations of MDMA were present in the white matter. This is in accordance with toxicological data obtained in methamphetamine users [29]. The discrepancy between the substantial concentrations in the white and the grey matter, as detected by GC-MS and LC-MS, and the relatively weak immunoreactivity of the white matter in the brain lobes, may be explained by the fact that in the latter approach MDMA can only be detected if it is bound to the tissue and that this complex is not affected by the procedure of fixation, paraffin embedding and sectioning. We presume that unbound MDMA is washed out during the immunohistochemical preparation procedure. In the tissue homogenates used for the GC-MS and LC-MS analyses, both the bound MDMA and the soluble form remain available for detection. For methamphetamine as well, positive immunoreactivity in the cerebral cortex and in the white matter was demonstrated [24]. In our study, topographic differences in staining intensity were observed. In particular, distinct immunoreactivity was found in the neurons of all cortical brain regions, the basal ganglia and the cerebellum. However, the neuron cell bodies in the brainstem were relatively weakly stained and the white matter fibres were more pronounced at that site. In addition, immunoreactivity was also found in the cells of the dentate gyrus in the hippocampus and the granular layer cells of the cerebellar vermis. This was not observed in the immunohistochemistry of morphine and methadone [13, 14].

The observed results in our two fatalities were well consistent with the topographic data obtained in rats after injection of [^{3}H]-MDMA and [^{3}H]-MDA [18]. We were able to provide further information about the regional distribution of MDMA and MDA in human brain tissue. In addition, the method may be used to further investigate the biological effects, for example the interaction of MDMA and MDA on serotonin systems which was previously demonstrated [18, 30], to study the influence on memory [31] etc.

Immunostaining of the pituitary gland revealed a variable reaction intensity in the different cells (acidophils, basophils and chromophobes). The most intensely stained cells were probably the acidophils as these are metabolically the most active cells. This is in accordance with the MDMA-induced neuroendocrine effects such as increases in plasma cortisol and prolactin levels which have been documented in humans [32]. We will undertake studies to correlate the heterogeneity of the MDMA reactivity with the hormone producing cell types.

Although the mechanism of death in our two fatalities was different, cardiopulmonary failure related to the sympaticomimetic effects of MDMA, and DIC due to hyperthermia, respectively and although there were obvious differences in toxicology data, the immunoreactivity pattern in the brain regions of both cases was fairly comparable. Despite the hyperthermia and the post-mortem interval of about 3 days in the second case leading to a more pronounced tissue autolysis, the drugs could be immunohistochemically demonstrated. However, it should be kept in mind that interfering factors such as putrefaction may give rise to false negative results. On the contrary, it cannot be excluded that, theoretically, the putrefaction process might lead to artefactual epitopes which are recognised immunologically and thus could induce a false positive reaction. However, in the control cases (with post-mortem interval up to 2 days) no reaction was observed, except for a background level.

In the cases and controls no immunoreactivity was found after incubation with IgG_1 fraction from normal serum. In the controls a very faint diffuse staining was found in a few neurons after incubation with the anti-MDMA antibodies. Given the fact that saturation of the antibody with pure MDMA abolished staining in the cases and that in the controls, no amphetamines could be detected by chromatographic analysis, the very weak diffuse reaction in some neurons of the controls must be considered as the background level. A similar phenomenon of diffuse staining in control neurons was experienced by Wehner et al. [13, 14] in their study on immunodetection of methadone and morphine in human brain. As emphasised by these authors, it must be kept in mind that the CSA detection method yields an extreme amplification of the primary signal (antigen-antibody complex) and that also the slightest background becomes enhanced as a result of the sensitivity of the method.

Since saturation of the antibody with either MDMA or MDA reduced staining to background level, the specificity of the antibodies and the method was confirmed. According to the information obtained by the supplier, the antibodies are highly specific for ecstasy (MDMA) and related compounds (MDA and MDEA) while cross-reaction with amphetamine, methamphetamine and some medications (phentermine, phenyl propanolamine, pseudoephedrine) is always below 1% (pers. comm. Mr R. Ramage, Microgenics Corp., Fremont, Calif.).

In summary, a reliable method for the specific immunohistochemical detection of MDMA and MDA in human brain tissues is presented. This method can be used as evidence of intake or even poisoning with MDA, MDMA and/or MDEA and can be a reliable alternative when the usual samples (blood and urine) are not available for toxicological analysis, for example due to fulminant blood loss in severely destroyed bodies, such as polytrauma in train accident fatalities.

Further studies will be undertaken in order to investigate whether this technique might be influenced by post-mortem processes. In addition, MDMA immunodetection might be used as a basis for further study of the distribution of these amphetamine analogues in the human body, and thus be useful in the understanding of their biological effects.

Acknowledgments The authors wish to thank Microgenics Corp, Fremont, CA, USA and Mr R Ramage, PhD, Sr. Scientist in particular, for their advice and support and for providing the appro-

priate antibodies. We also thank Mrs Thérèse De Vuyst for her assistance in preparing the manuscript and Mr G. Van Limbergen for technical assistance.

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