Int J Legal Med (2000) 113 : 201–207

ORIGINAL ARTICLE

Yoshitaka Maeno · Mineo Iwasa · Hiroyuki Inoue
Hiroyoshi Koyama · Ryoji Matoba

Methamphetamine induces an increase in cell size and reorganization of myofibrils in cultured adult rat cardiomyocytes

Received: 1 February 1999 / Received in revised form: 1 July 1999

Abstract To investigate the direct effects of methamphetamine (MAP) on cardiac lesions seen in MAP abusers, isolated adult rat ventricular cardiomyocytes (ARCs) were exposed to MAP (0.05–1.0 mM) in medium 199 containing 10% fetal calf serum. Isolated ARCs attached to laminin-coated substrata and began to spread into polygonal shapes with pseudopodia at day 6 in normal culture. However, the cell attachment and spreading were inhibited by exposure to MAP (0.5 and 1.0 mM) for the first 7 days in culture. On the other hand, exposure to MAP (0.05 and 0.1 mM) for 7 days after a 6-day period of normal culture, led to a larger cross surface area of cells with more abundant actin bundles compared to control cells ($p < 0.05$). This development of spreading area resembled that of norepinephrine-treated ARCs. In addition, immunoreactive atrial natriuretic peptide (ANP) granules developed and accumulated around the nuclear region of ARCs exposed to MAP and the number of ANP positive cells tended to increase in a dose-dependent manner. These results suggest that chronic exposure to a high concentration of MAP may directly inhibit development of ARCs in culture and that a continuous exposure to a low concentration of MAP may facilitate the development of cellular hypertrophy. Therefore, hypertrophied cardiomyocytes in MAP abusers may be provoked by multifactorial incidents of direct and indirect actions of MAP.

Key words Methamphetamine · Cardiotoxicity · Cardiomyocyte · Cell culture · Hypertrophy

Y. Maeno (✉) · M. Iwasa · H. Inoue · H. Koyama
Department of Legal Medicine,
Nagoya City University Medical School, Kawasumi 1,
Mizuho-ku, Nagoya 467–8601, Japan
e-mail: maenoyo@med.nagoya-cu.ac.jp,
Tel.: +81-52-8538181, Fax: +81-52-8427701

R. Matoba
Department of Legal Medicine,
Osaka University Medical School, Yamadaoka 2–2, Suita,
Osaka 565–0871, Japan

Introduction

The number of methamphetamine (MAP) abusers has risen again since the 1970s after initially decreasing in the 1960s and a number of sudden deaths in MAP abusers have also been observed. MAP has been classified as an indirectly acting sympathomimetic amine such as phenylisopropylamines including ephedrine, amphetamine (AMP) and tyramine. It facilitates liberation of norepinephrine from adrenergic nerve terminals and inhibits its re-uptake (Hoffman and Lefkowitz 1996). Thus, users will be in a hyperactive, manic, stimulated or euphoric state, characterized by increased mood and libido, depressed appetite and often by confusion and delusions (Caldwell and Sever 1974). Since Smith et al. (1976) reported a case of cardiomyopathy induced by dextroamphetamine in a patient who abused the drug, it has been known that overdose or chronic administration of amphetamines including MAP (AMPs) induce various cardiac lesions which might lead to sudden death. In acute poisoning by AMPs, the major histological changes of the myocardium are eosinophilic degradation, myocardial fiber necrosis with myoglobin loss, cellular infiltration, contraction bands and swollen mitochondria (Call et al. 1982; Kaiho and Ishiyama 1989; Katsumata et al. 1993; Maruta et al. 1997) and MAP overdose results in cardiovascular manifestations such as tachycardia, atrioventricular arrhythmia, myocardial ischemia and hypertension, thereby causing sudden acute congestive heart failure and death (Derlet and Horowitz 1995). In addition, cardiac hypertrophy, disarrangement of myofibers, rupture of the myocardium and fibrosis have been observed in chronic AMP abusers and it has been indicated that these alterations of the myocardium were commonly seen in hypertrophic cardiomyopathy (Smith et al. 1976; Matoba et al. 1984; Fukunaga et al. 1987; Tanaka et al. 1989; Hong et al. 1991; Islam et al. 1995; He et al. 1996). Smith et al. (1976) reported that cardiomyopathy was associated with chronic AMP ingestion and suggested that this might be the end-result of chronic myocardial hypertrophy induced by AMP. Fur-

thermore, Matoba et al. (1994) reported that immunoreactive granules of atrial natriuretic peptide (ANP) which is known as a fetal protein, were observed in the ventricles of MAP abusers. This peptide is primarily secreted from atrial myocytes in response to local wall stretch and the ventricular re-expression is recognized as a hypertrophic reaction (Eppenberger-Eberhardt et al. 1993). It has been discussed that some of the cardiac lesions found in AMP abusers are pathological findings mediated indirectly by peripheral catecholamines released from nerve terminals by AMP administration (Uchima et al. 1983; Cho 1990). However, a direct cardiotoxicity of AMPs is not clear. Although there are some recent reports that in vitro MAP directly elicits cardiotoxicity during short-term exposure in isolated adult rat ventricular cardiomyocytes (ARCs) (Welder 1992; He 1995), a direct effect of chronic exposure to MAP on morphological changes of cardiomyocytes has not been described. Piper et al. (1990) described that isolated cardiomyocytes represent a useful model for studies on cardiac metabolism and function and cultures may be interesting models to investigate the molecular mechanism of cardiac hypertrophy. The aim of this study was to investigate isolated ARCs in culture to show whether MAP directly influenced cardiac lesions such as seen in MAP abusers, especially hypertrophic cardiomyocytes and the ventricular expression of ANP.

Materials and methods

Preparation of myocytes

Freshly isolated adult cardiomyocytes were prepared from 8-week-old male Wistar rats according to the modified protocol of Piper et al. (1990). Briefly, animals were anesthetized with pentobarbital and after injecting heparin (300 IU/kg) into the inferior vena cava, the heart was excised and the aorta was cannulated. The heart was perfused retrogradely with a modified Ca^{2+}-free perfusion buffer containing 110 mM NaCl, 2.6 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 11 mM Glucose and 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (at 37 °C, pH 7.4, gassed with 95% O_2-5% CO_2, for 5 min.). Then perfusion was switched to 0.03% collagenase solution (CLS 2, Worthington Biochemical) containing 0.004% pronase (Boehringer Mannheim), 0.005% trypsin (Sigma, 1 : 250) and 0.04 mM $CaCl_2$ in perfusion buffer. After perfusion with collagenase solution for 20 min, the ventricles were minced in the collagenase solution containing 1.2% bovine serum albumin (BSA fraction V, Gibco). After incubating at 37 °C for 10 min in the collagenase solution, the myocytes were filtered through a nylon mesh and centrifuged at $8 \times g$ for 3 min and the pellet was washed in perfusion buffer containing 0.1 mM $CaCl_2$. After separation with a 33% Percoll (Pharmacia) separation medium containing 140 mM NaCl, 5 mM KCl, 0.8 mM $MgCl_2$, 0.82 mM Na_2HPO_4, 0.37 mM NaH_2PO_4, 12.4 mM glucose, 18.7 mM HEPES, 0.2 mM $CaCl_2$ and 0.034% BSA, myocytes were washed with perfusion buffer containing $CaCl_2$ which was added stepwise to a concentration of 1.0 mM. The cells were suspended in medium 199 (Sigma, M199) containing 5 mM creatine, 2 mM L-carnitine, 5 mM taurine, 0.1 mM insulin, 10 mM cytosine β-D-arabino-furanoside, 100 IU/ml penicillin-streptomycin and 10% fetal calf serum (FCS, Gibco). Cardiomyocytes were plated on culture chamber slides (Nunc, Naperville, Ill.) coated with 5 mg/ml laminin (Collaborative Biomed. Products) at 3×10^3 cells/well and incubated in a CO_2 incubator at 37 °C. The proportion of rod-shaped cells was approximately 90–95% by phase contrast microscopy. After 3 h, the medium was replaced with fresh medium containing 10% FCS and myocytes were cultured until drug exposure. All animal protocols were approved by the Committee for experiments with animals at the Nagoya City University Medical School in Japan.

Protocol of drug treatment

This protocol consists of exposure to MAP at two stages in the culture of ARCs. Generally, it has been known that ARCs undergo a slow morphological transition in culture, which are divided into two phases: freshly isolated myocytes attach to the substratum and change from a rod-shaped cell to a round-shaped cell in the early stage of culture. The transition to these rounded cells is accompanied by the loss of myotypic structures and organelles such as myofibrils (Guo et al. 1986; Eppenberger et al. 1994; Horackova and Byczko 1997). This stage is called dedifferentiation in culture. Thus, the first treatment with MAP was carried out from 0–7 days in culture and freshly isolated cardiomyocytes were exposed to 0.1, 0.5 and 1.0 mM MAP-HCl (Dainippon Pharmaceutical, Osaka, Japan) dissolved in culture medium and then the medium containing each concentration of MAP was changed to chronic expose to MAP every 2 days up to day 7 in culture (Protocol 1). The second phase is called redifferentiation in culture where dedifferentiated myocytes begin to spread out from 5 or 6 days after cell plating and then myocytes grow without cell division. In this stage, the cytoskeletal structures which decreased during dedifferentiation in culture are reorganized. This stage in culture is suitable for the studies on the mechanism of cardiac hypertrophy (Piper et al. 1990). Therefore, myocytes were exposed to 0.05, 0.1 and 0.5 mM MAP for 7 days after the first 6-day normal culture (Protocol 2). Medium with or without MAP was changed every 2 days in culture.

Immunocytochemistry

In order to observe morphological changes of cultured cells, cells were fixed with 4% paraformaldehyde, permeabilized with 0.05% Triton X-100 (Wako, Japan) and reacted with tetramethylrhodamine B isothiocyanate (TRITC)-conjugated phalloidin (Sigma) for cytoskeletal actin (F-actin) staining. For immunolabeling of atrial natriuretic peptide (ANP) in myocytes, a rabbit anti-ANP polyclonal antibody (Peptide Inst., Japan) and biotin-conjugated antibody (Donkey anti-rabbit IgG H+L, Jackson ImmunoResearch) were used as first and second antibodies, respectively. To enhance the sensitivity of immunolabeling, fluoroisothiocyanate (FITC)-conjugated streptavidin (Vector) was incubated as the final step. Samples were scanned by a laser confocal scanner (BioRad, MRC-1000) equipped with a Zeiss fluorescence microscope (Carl Zeiss).

Cell size

Estimation of cell size was conducted on cultures following fixation and staining with TRITC-conjugated phalloidin. The largest cross surface area of a cell was measured using an image analytical system (BioRad, COMOS). Cell images stained with TRITC-conjugated phalloidin were studied with a $10 \times$ magnification objective. The number of cells was counted on a fixed field of 730,136.12 μm^2 and then the total area of cells was measured. The number of cells per fixed field was around 40–100 cells. Cell size was calculated as a quotient of the total cell area and the cell numbers. Myocyte size was determined in five randomly selected fields per culture.

Statistical evaluation

The results were expressed as the mean ± SD and Student's *t*-test was used for evaluation of significance. Values of $P < 0.05$ were considered to be significant.

Results

Influence of MAP exposure on morphological transition of cultured myocytes during dedifferentiation in culture (Protocol 1)

Freshly isolated rod-shaped ARCs attached to a substratum and then became rounded within the first 2–3 days in culture containing 10% FCS. This phase is a period of dedifferentiation of cultured ARCs. Afterward, some of the round-shaped cells began to spread out after 5–6 days culture. After 7 days in culture, ARCs transformed into a polygonal shape with pseudopodia-like structures which were filled with abundant actin stress fiber-like structures (Fig. 1 A). In MAP-treated cells, the shapes of cells exposed to 0.1 mM MAP for the first 7 days in culture, were similar or slightly larger in size than untreated cells, while the number of cells treated with 0.5 and 1.0 mM MAP decreased remarkably and they remained as round-shaped cells (Fig. 1 B-D). Therefore, a concentration higher than 0.5 or 1.0 mM MAP adversely influenced the morphological transition of ARCs such as cell attachment and cell spreading during the first 7 days in culture.

Effect of MAP on myocyte spreading and growth during redifferentiation in culture (Protocol 2)

After a long-term culture in medium containing 10% FCS, ARCs began to spread after dedifferentiation and then the cell size gradually increased with extending and elongating pseudopodia-like structures containing actin stress fibers. In 13-day-old ARCs in culture medium without addition of MAP, most of the cells spread out and some cells connected with the pseudopodia. Furthermore, a few striated myofibrils appeared in the central part of the cell (Fig. 2 A). This phase is called redifferentiation. In MAP-treated groups, cultured myocytes that were exposed to 0.05 and 0.1 mM MAP for 7 days (day 6–13) were larger than untreated control cells. On day 7 (day 13 in culture) after exposure to MAP, the spreading area of the cell was more extended and the pseudopodia-like structures elongated until they contacted with one another and the contact area also increased. Actin filaments stained with phalloidin were longitudinally organized along the spreading direction of the cell and cell margin, in particular many radially striated actin filament bundles were seen in the central part of cells exposed to 0.1 mM MAP (Fig. 2 B and C). However, after treatment with 0.5 mM MAP, the cell shapes were different from that of untreated control cells and were characterized by a broom-like extension and shortening pseudopodia-like structures, although actin filament bundles were observed in the central part of the cell (Fig. 2 D).

It has been reported that cardiac hypertrophy characterized by an increase in cell size was induced by catecholamines (Simpson et al. 1982; Simpson and McGrath 1983; Simpson 1985; Tang et al. 1987; Clark et al. 1991). Therefore, to confirm whether cellular hypertrophy occurred in ARCs used in this experiment, ARCs were exposed to 0.1, 1.0 or 2.0 μM *l*-norepinephrine bitartrate (NE, Wako, Japan) for 7 days (from days 6 to 13 in culture). After 7 days (day 13 in culture) exposure to NE, cell spreading with polygonal pseudopodia-like structures filled with actin filament bundles increased until they contacted with one another and the striated actin filament bundles were more obvious in the central part of the cell compared with control cells (Fig. 3 A-C). These results in-

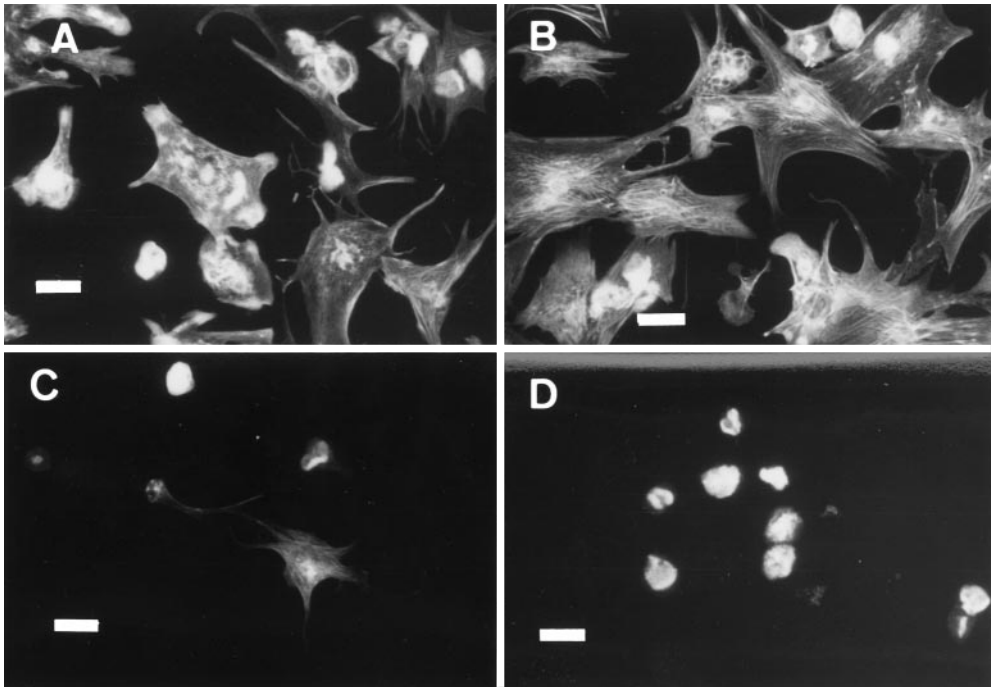

Fig. 1 A–D Confocal micrographs of adult rat cardiomyocytes (ARCs) after 7 days in 10% fetal calf serum (FCS) supplemented culture with or without addition of methamphetamine (MAP) in protocol 1 described in Materials and methods, labeled for F-actin with TRITC-phalloidin. **A** Untreated control ARCs at 7 days in culture, **B**, **C** and **D** treated with 0.1, 0.5 and 1.0 mM MAP for 7 days, respectively. Control and treated cells with 0.1 mM MAP showed spreading with pseudopodia-like structures. Cell attachment and spreading were inhibited by exposing to 0.5 and 1.0 mM MAP during dedifferentiation. Scale bar is 50 μm

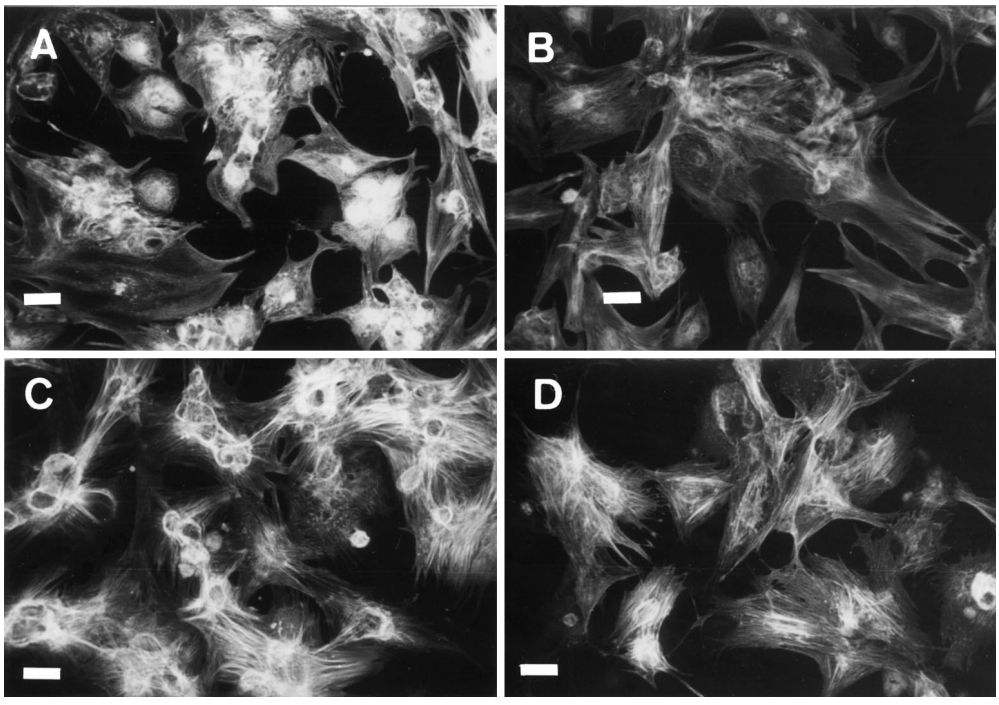

Fig. 2 A–D Confocal micrographs of 13-day-old ARCs in culture with or without addition of MAP in protocol 2, labeling for F-actin with TRITC-phalloidin. **A** Untreated control ARCs at day 13 in culture, **B** 0.05 mM, **C** 0.1 mM, **D** 0.5 mM MAP exposure for 7 days after 6-day normal culture. ARCs exposed to 0.05 and 0.1 mM MAP showed wider spreading and more abundant actin fibers than control cells. Scale bar is 50 μm

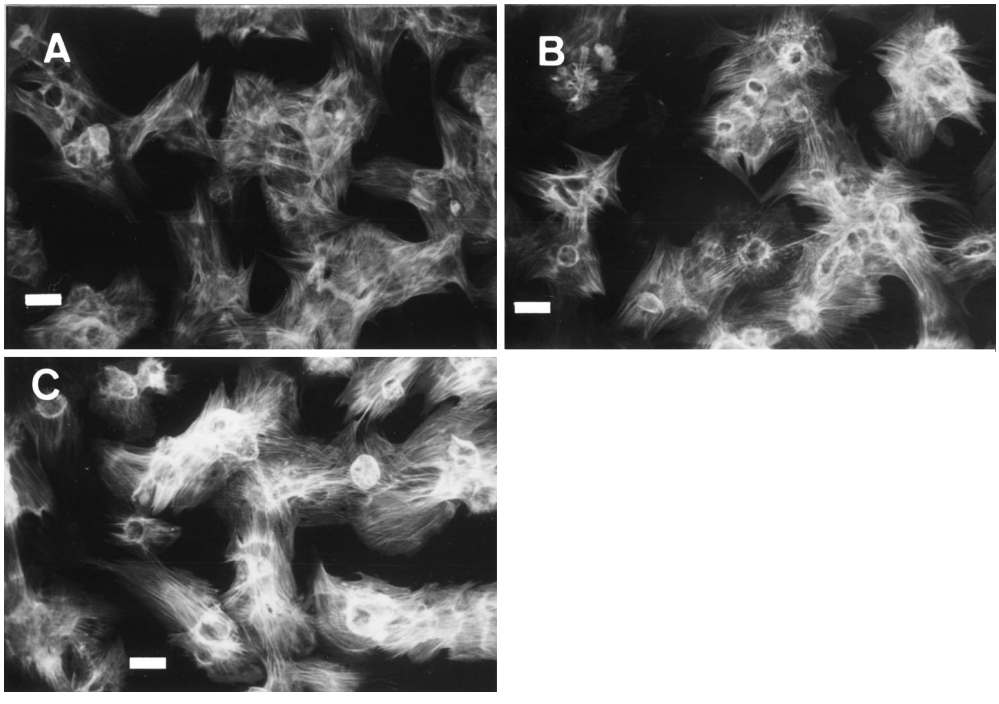

Fig. 3 Confocal micrographs of ARCs exposed to **A** 0.1, **B** 1.0 and **C** 2.0 μM norepinephrine (NE) for 7 days after 6-day normal culture (protocol 2), labeling for F-actin with TRITC-phalloidin. More extensive spreading with abundant actin bundles were seen to be dose-dependent compared to control cells (see Fig. 2). Scale bar is 50 μm

dicated that the 6-day-old ARCs used in this experiment functioned against ligands to provoke cellular hypertrophy. Thus, to estimate those morphological changes such as cell spreading and growth in the presence of MAP, cell size was determined at a position close to the substratum in culture using an image analytical system equipped with a laser confocal microscope MRC-1000. As shown in Fig. 4, although the largest cross surface area of ARCs treated and untreated with MAP or NE increased progressively in a time and dose-dependent manner, treated cell groups were significantly larger than untreated control groups except for 0.5 mM MAP-treated cells. In particular, the areas of the treated cells which were exposed to 0.05 and 0.1 mM MAP at 7 days after a 6-day normal culture (day 13 in culture) were 7,001.5 ± 2,140.8 and 7,103.4 ± 1,552.0 μm^2/cell, respectively and were on average 1.7-fold larger than the area of the untreated control cells (4,202.2 ± 1,349.3 μm^2/cell) after the same time. After treatment with 0.1, 1.0 and 2.0 μM NE, the area also increased in a time and dose-dependent manner to 6,951.4 ±

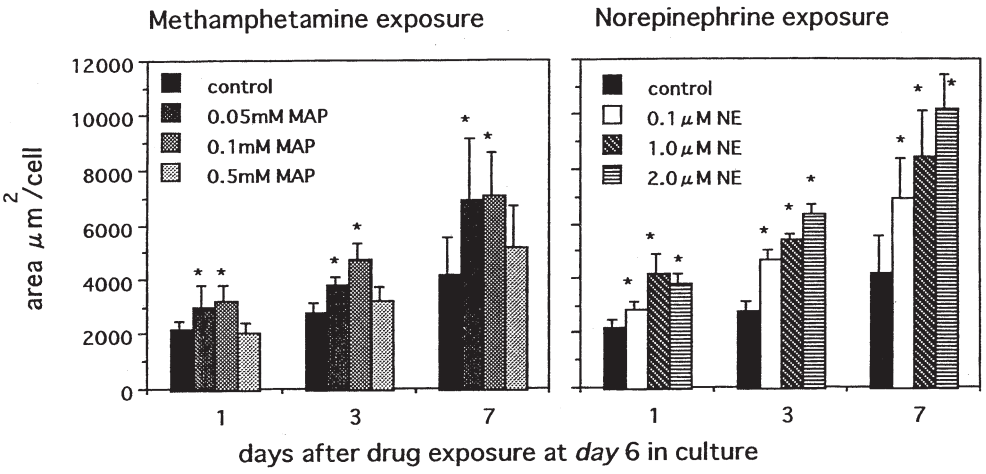

Fig. 4 Changes in the maximum transversal area of spreading ARCs exposed to MAP or NE for 7 days after 6-day normal culture. ARC areas increased time and dose-dependently with exposure to MAP or NE, except for 0.5 mM MAP treatment. Data represent the mean ± SD from five different fields. *$P < 0.05$ versus control

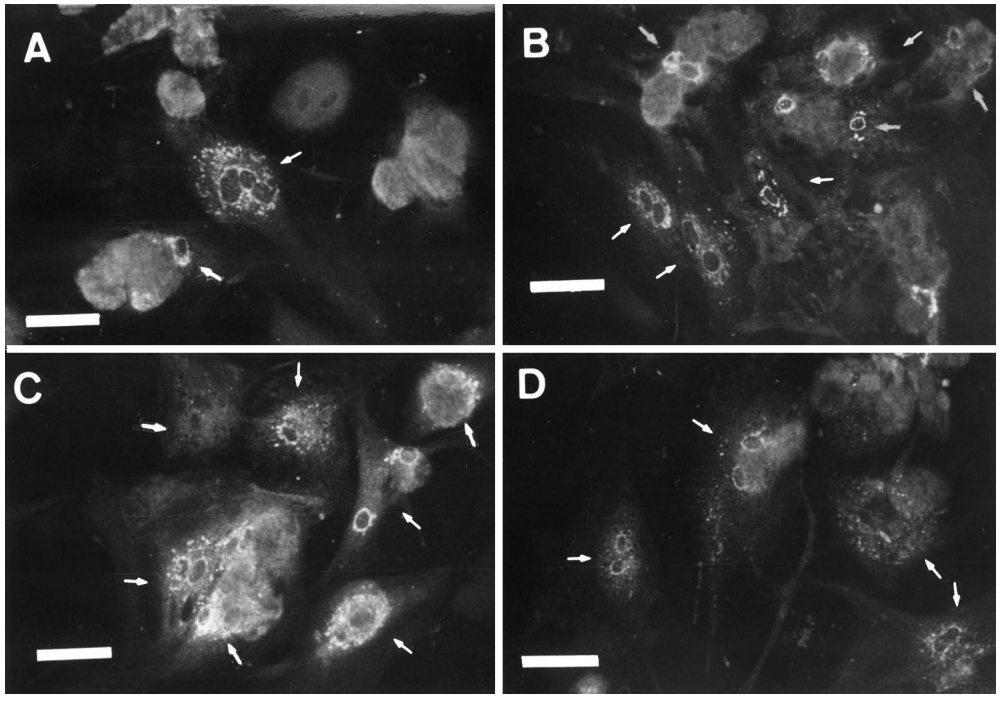

Fig. 5 A–D Confocal micrographs of ARCs exposed to MAP for 7 days after 6-day normal culture (protocol 2), labeling with a polyclonal antibody against atrial natriuretic peptide (ANP). **A** Untreated control cells (day 13 in culture), **B** 0.05, **C** 0.1, **D** 0.5 mM MAP exposure for 7 days (day 13 in culture). Note accumulation of immunoreactive ANP granules around the nucleus of the spreading ARCs and increases in the number of ANP positive cells (*arrows*) with rising concentration of MAP. Scale bar is 50 μm

1,444.6 (1.7-fold), 8,513.3 ± 1,616.9 (2.0-fold) and 10,269.9 ± 1,142.5 μm^2/cell (2.4-fold area), respectively ($p < 0.05$).

Expression of atrial natriuretic peptide (ANP) in myocytes by MAP treatment

In addition to facilitation of cell spreading and growth in this experiment, it was an important event of cellular hypertrophy that the ventricular re-expression of ANP and the formation of granules were observed in cultured ARCs exposed to MAP. As shown in Fig. 5, immunoreactive ANP granules were strictly confined to the perinuclear region of spreading cells and more ANP positive cells were seen in the treatment groups with 0.05, 0.1 and 0.5 mM MAP for 7 days after 6-day normal culture as compared with untreated control cells on day 13 in culture.

Discussion

In this study we describe the direct effects of chronic MAP exposure on morphological alterations of ventricular cardiomyocytes using cultured ARCs in the presence of 10% serum in vitro. The effects seem to relate to cellular hypertrophy of ARCs.

Generally, isolated ARCs undergo morphological changes in FCS-supplemented culture. Freshly isolated ARCs attached to the substratum and most cells become spherical up to at least 3–4 days in culture. This stage has been termed dedifferentiation in culture and it is required for the next stage redifferentiation in culture, namely cel-

lular spreading and growth (Claycomb and Palazzo 1980; Guo et al. 1986; Piper et al. 1990; Eppenberger et al. 1994; Horackova and Byczko 1997). In this experiment, isolated ARCs were cultured in medium containing 10% FCS to investigate the effect of long-term exposure to MAP on the morphological transition of ARCs. The cultured myocytes exposed to 0.5 and 1.0 mM MAP for the first 7 days (in protocol 1) failed to spread out and the number of cells remaining on the culture substratum after staining decreased. These results indicate that MAP inhibits the onset of the morphological transition of ARCs in culture. Salomon (1978) demonstrated that AMP inhibited synthesis of cytoskeletal proteins. Tubulin and actin were inhibited to a very small degree but myosin synthesis was markedly reduced in cultured chick myotubes. In addition, microtubular structures closely associated with actin always presented in an organized network and were necessary for cell attachment during dedifferentiating ARCs in normal culture (Guo et al. 1986). Therefore, the MAP-induced morphological changes of ARCs for the first 7 days in culture in this study may be due to inhibition of protein synthesis or degeneration of microtubule structures in ARCs.

Moreover, we have observed the time and dose-dependent facilitation of cell spreading with pseudopodia-like structures in MAP-treated cells (in Protocol 2). The cell size when exposed for 7 days to 0.05 and 0.1 mM MAP after 6-day normal culture was 1.7-fold larger than that of untreated cells at day 13 in culture, although the size of untreated cells also gradually increased. In addition, the area of cell-cell contact also increased and F-actin structures stained with phalloidin radially developed with striated thick fibers in the center of the cell when ARCs were exposed to 0.1 and 0.5 mM MAP for 7 days after 6-day normal culture. Simpson and colleagues (1982; 1983; 1985) demonstrated that the myocardial cell size evaluated by cell volume, surface area and total protein was regulated by variation in culture media, including serum and catecholamines. These regulating factors increased in a dose-dependent manner with the cell size, indicating that they induce myocardial cell hypertrophy. In the culture model used in the present study, NE stimulated time and dose-dependent cellular hypertrophy such as an increase in cell size and reorganization of myofibrils in redifferentiated ARCs in culture and MAP-induced morphological changes in cultured ARCs closely resembled NE-stimulated cellular hypertrophy. These results suggest that MAP is likely to possess hypertrophic effects which are nearly comparable to NE. It has been known that NE-stimulated cellular hypertrophy of isolated neonatal or adult rat cardiomyocytes was mediated through an α1-adrenoreceptor (Simpson and McGrath 1983, Simpson 1985; Piper et al. 1988; Volz et al. 1991). In this study, the effect of 0.05 mM MAP on an increase in cell size was approximately comparable to 0.1 μM NE. We therefore suspect, that although an action site of MAP on cultured ARCs has not yet been confirmed, if the action site is the α-adrenergic receptor, the affinity of MAP for the receptor may be weaker than that of NE.

On the other hand, ARCs exposed to 0.5 mM MAP failed to show an increase in the area of cell spreading although they contained actin bundle structures, but not as abundantly as the cells treated with 0.1 mM MAP. This may indicate that a concentration of MAP higher than 0.5 mM reduces rather than increases cell spreading or protein synthesis of the redifferentiated ARCs.

To further support the MAP-promoted cellular hypertrophy, we carried out immunocytochemical staining of ANP. This peptide is one of the fetal proteins (Izumo et al. 1987; Woodcock-Mitchell et al. 1989; Eppenberger-Eberhardt et al. 1993), re-expressed during a hypertrophic reaction in cardiomyocytes. Under normal conditions in the mammalian heart, ANP exists predominantly in the atrium and the levels are approximately 100-fold greater than ventricular levels (Gardner et al. 1986), whereas ventricular expression of ANP increases with conditions of severely altered hemodynamic load (Ruskoaho 1992). In the present study, immunoreactive granules of ANP were localized around the nuclei in some spreading ARCs on day 13 in normal culture. This localization is consistent with that of 7-day-old ARCs in culture containing 20% FCS as described by Eppenberger-Eberhardt et al (1993). However, the ANP immunostaining in ARCs treated with MAP for 7 days after 6-day normal culture was stronger than that of untreated control cells and the number of ANP positive cells increased with MAP exposure. These results suggest that the treatment with MAP facilitates upregulation of ANP expression in cultured ventricular myocytes with increased cell area and seems to support the suggestion that ARCs undergo hypertrophy. Knowlton et al. (1991) reported that α1-adrenergic agonists could activate ANP gene expression in neonatal rat ventricular myocytes. Therefore, the appearance of immunoreactive ANP in the ventricles of MAP abusers (Matoba et al. 1994) may result not only from a hemodynamic load provoked by MAP-induced excessive liberation of catecholamines but also by direct action of MAP on ANP expression.

In conclusion, the results of this study suggest that MAP has a direct effect on myocytes concerning increase in cell size and ANP re-expression using cultured ventricular cardiomyocytes of adult rats. A chronic exposure to high concentrations of MAP reduced cell spreading and attachment, whereas low concentrations of the drug facilitated an increase in cell size, reorganization of myofibrils and ANP re-expression. Therefore, hypertrophied cardiomyocytes seen in MAP abusers may be provoked by not only by indirect but also by direct actions of MAP. Further studies are required to determine the action site of MAP that mediate cellular hypertrophy.

Acknowledgments The authors would like to thank Prof. Dr. Jutta Schaper and Dr. Sawa Kostin, Department of Experimental Cardiology Max Planck Institute, for their helpful suggestions on the methods of cell culture and immunocytochemistry.

References

Caldwell J, Sever PS (1974) The biochemical pharmacology of abused drugs I. Amphetamines, cocaine and LSD. Clin Pharm Ther 16:625–638

Call TD, Hartneck J, Dickinson WA, Hartman CW, Bartel AG (1982) Acute cardiomyopathy secondary to intravenous amphetamine abuse. Ann Intern Med 97:559–560

Cho AK (1990) Ice: A new dosage form of an old drug. Science 249:631–634

Clark WA, Rudnick SJ, Lapres JJ, Lesch M, Decker RS (1991) Hypertrophy of isolated feline heart cells following β-adrenergic-induced beating. Am J Physiol 261:C530-C542

Claycomb WC, Palazzo MC (1980) Culture of the terminally differentiated adult cardiac muscle cell: a light and scanning electron microscope study. Dev Biol 80:466–482

Derlet RW, Horowitz BZ (1995) Cardiotoxic drugs. Emerg Med Clin N Am 13:771–791

Eppenberger HM, Hertig C, Eppenberger-Eberhardt M (1994) Adult rat cardiomyocytes in culture: a model system to study the plasticity of the differentiated cardiac phenotype at the molecular and cellular levels. Trends Cardiovasc Med 4:187–193

Eppenberger-Eberhardt M, Messerli M, Eppenberger HM, Reinecke M (1993) New occurrence of atrial natriuretic factor and storage in secretorially active granules in adult rat ventricular cardiomyocytes in long-term culture. J Mol Cell Cardiol 25: 753–757

Fukunaga T, Mizoi Y, Adachi J, Tatsuno Y, Fujiwara S, Ueno Y (1987) Methamphetamine concentrations in blood, urine, and organs of fatal cases after abuse. Nippon Hoigaku Zasshi 41: 328–334

Gardner DG, Deschepper CF, Ganong WF, Hane S, Fiddes J, Baxter JD, Lewick J (1986) Extra-atrial expression of the gene for atrial natriuretic factor. Proc Natl Acad Sci USA 83:6697–6701

Guo J-X, Jacobson SL, Brown DL (1986) Rearrangement of tubulin, actin, and myosin in cultured ventricular cardiomyocytes of the adult rat. Cell Motil Cytoskel 6:291–304

He S-Y (1995) Methamphetamine-induced toxicity in cultured adult rat cardiomyocytes. Nippon Hoigaku Zasshi 49:175–186

He S-Y, Matoba R, Sodesaki K, Fujitani N, Ito Y (1996) Morphological and morphometric investigation on cardiac lesions after chronic administration of methamphetamine in rats. Nippon Hoigaku Zasshi 50:63–71

Hoffman BB, Lefkowitz RJ (1996) Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonists. In: Hardman JG, Limbird LE, (eds) Goodman and Gilman's the pharmacological basis of therapeutics, 9th edn. McGraw Hill, New York, pp 199–248

Hong R, Matsuyama E, Nur K (1991) Cardiomyopathy associated with the smoking of crystal methamphetamine. JAMA 265: 1152–1154

Horackova M, Byczko Z (1997) Differences in the structural characteristics of adult guinea pig and rat cardiomyocytes during their adaptation and maintenance in long-term cultures: confocal microscopy study. Exp Cell Res 237:158–175

Islam MN, Kuroki H, Hongcheng B, Ogura Y, Kawaguchi N, Onishi S, Wakasugi C (1995) Cardiac lesions and their reversibility after long term administration of methamphetamine. Forensic Sci Int 75:29–43

Izumo S, Lompre AM, Matsuoka R, Koren G, Schwartz K (1987) Myosin heavy chain messenger RNA and protein isoform transitions during cardiac hypertrophy: interaction between hemodynamic and thyroid hormone-induced signals. J Clin Invest 79: 970–977

Kaiho M, Ishiyama I (1989) Morphological study of acute myocardial lesions experimentally induced by methamphetamine. Nippon Hoigaku Zasshi 43:460–468

Katsumata S, Sato K, Kashiwade S, Yamanami S, Zhou H, Yonemura I, Nakajima H, Hasekura H (1993) Sudden death due presumably to internal use of methamphetamine. Forensic Sci Int 62:209–215

Knowlton KU, Baracchini E, Ross RS, Harris AN, Henderson SA, Evans SM, Glembotski CC, Chien KR (1991) Co-regulation of the atrial natriuretic factor and cardiac myosin light chain-2 genes during alpha-adrenergic stimulation of neonatal rat ventricular cells. Identification of cis sequences within an embryonic and a constitutive contractile protein gene which mediate inducible expression. J Biol Chem 266:7759–7768

Maruta T, Nihira M, Tomita Y (1997) Histopathological study on acute poisoning of methamphetamine, morphine or cocaine. Jpn J Alcohol Drug Depend 32:122–138 (in Japanese)

Matoba R, Onishi S, Shimizu Y, Shikata I (1984) Sudden death in methamphetamine abusers: A histological study of the heart. Nippon Hoigaku Zasshi 38:199–205 (in Japanese)

Matoba R, Fujitani N, Onishi S, He S-Y, Maeno Y (1994) Appearance of atrial natriuretic peptide in the ventricles of methamphetamine abusers. In: Nagano M, Takeda N, Dhalla NS (eds) The adapted heart. Raven Press, New York, pp 455–466

Piper HM, Jacobson SL, Schwartz P (1988) Determinants of cardiomyocyte development in long-term primary culture. J Mol Cell Cardiol 20:1081–1085

Piper HM, Volz A, Schwartz P (1990) Adult ventricular rat heart muscle cells. In: Piper HM (ed) Cell culture techniques in heart and vessel research. Springer, New York, pp 36–60

Ruskoaho H (1992) Atrial natriuretic peptide: synthesis, release, and metabolism. Pharmacol Rev 44:479–602

Salomon R (1978) The effect of amphetamines on culture myotubes: selective inhibition of protein synthesis. Life Sci 23: 1941–1950

Simpson P (1985) Stimulation of hypertrophy of cultured neonatal rat heart cells through an α1-adrenergic receptor and induction of beating through an α1- and β1- adrenergic receptor interaction. Circ Res 56:884–894

Simpson P, McGrath A (1983) Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an alpha 1 adrenergic response. J Clin Invest 72:732–738

Simpson P, McGrath A, Savion S (1982) Myocyte hypertrophy in neonatal rat heart cultures and its regulation by serum and by catecholamines. Circ Res 51:787–801

Smith HJ, Roche AH, Jagusch MF, Herdson PB (1976) Cardiomyopathy associated with amphetamine administration. Am Heart J 91:792–797

Tanaka Y, Nishi T, Chin M, Nagao M, Nishio T, Hashida E, Ito M, Kashiwagi A, Shigeta Y (1989) A case of hypertrophic cardiomyopathy associated with amphetamine abuse. Jpn Intern Soc Med 78:944–948 (in Japanese)

Tang Q, Taylor PB, Helbing RK (1987) Catecholamine induced cardiac hypertrophy. Can J Cardiol 3:311–316

Uchima E, Ogura Y, Hiraga Y, Shikata I (1983) Relationship between methamphetamine toxicity and catecholamine levels in heart and brain of mice. Nippon Hoigaku Zasshi 37:198–208 (in Japanese)

Volz A, Piper HM, Siegmund B, Schwartz P (1991) Longevity of adult ventricular rat heart muscle cells in serum-free primary culture. J Mol Cell Cardiol 23:161–173

Welder AA (1992) A primary culture system of postnatal rat heart cells for the study of cocaine and methamphetamine toxicity. Toxicol Lett 60:183–196

Woodcock-Mitchell J, Mitchell JJ, Low RB, Kieny M, Sengel P, Rubbis L, Skalli O, Jackson B, Gabbiani G (1989) α-Smooth muscle actin is transiently expressed in embryonic rat cardiac and skeletal muscles. Differentiation 39:161–166