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Molecular-biological analysis of the effect of methamphetamine on the heart in restrained mice

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ABSTRACT

In order to investigate the interaction in the heart between the administration of methamphetamine (MAP) and restraint of the body following it, we administrated MAP intraperitoneally to mice and restrained them, and then determined the level of mRNA expression of 22 genes in the heart using quantitative RT-PCR method. The mRNA expressions of Nfkbiz, Nr4a1 and Dusp1 changed significantly after the administration of MAP, suggesting the induction of an inflammatory condition such as damage to the myocardium. Moreover, the serum concentrations of inflammatory cytokines such as tumor necrosis factor-α, interleukin (IL)-1β and IL-6 were significantly increased by the administration of MAP. On the other hand, the mRNA expressions of Rgs2 and Rasd1 were changed by both the administration of MAP and body restraint without interaction, which indicated that these insults affected the circulatory system additively or synergistically. From these results, it is likely that the administration of MAP, followed by body restraint, might cause acute myocardial damage due to the direct myocardial toxic effect of MAP, myocardial hypoxia and/or severe hypertension, which is one of the mechanisms for sudden death in MAP abusers who were restrained due to their excited state.

1. Introduction

Methamphetamine (MAP) acts on both the central and sympathetic nervous systems, leading to euphoria and stimulation, which results in habitual use [1]. In Japan, the abuse of MAP is a matter of concern, and the number of abusers continues to increase gradually from year to year [2].

In the cases of MAP-related death, a forensic autopsy is performed to investigate the cause of death [3–5]. There have been autopsy cases where the individuals suddenly died immediately after being restrained due to excitement and/or delirium, and where MAP, at a concentration level less than fatal, was detected in the blood of the cadaver, but in which the mechanism of MAP-related death is still unclear. Stratton et al. [6] reported that, of 18 cadavers who suddenly died immediately after restraint due to excited delirium, 10 of them had some cardiac disease, including myocardial hypertrophy and fibrotic heart, and amphetamine, cocaine or marijuana were detected in the blood in 9 of the 10 cadavers. Moreover, it is known that, in forensic autopsies of MAP abusers, several cardiac lesions, such as myocardial hypertrophy, myocardial disarray and fibrosis of myocardium, are often observed [5,7–10]. Therefore, it is considered that MAP-related death may be related to these cardiac lesions or the restraint of the body.

In this study, in order to investigate the interaction in the heart between the administration of MAP and restraint of the body, we administered MAP to mice and/or restrained them, and then removed the heart. Subsequently, the change of the mRNA expression of many genes in the heart was determined using a microarray method and quantitative RT-PCR method, and we considered the molecular-biological mechanism of sudden death induced by the restraint of the body under the condition of MAP administration.

2. Materials and methods

The experiments were reviewed by the Committee of Ethics on Animal Experiments in the Faculty of Medicine, Mie University and carried out under the control of the Guideline for Animal Experiments in the Faculty of Medicine, Mie University and the Law (No. 105) and Notification (No. 6) of the Government of Japan. Moreover, this study followed the "Guideline for the Care and Use of Laboratory Animals" published by the US National Institute of Health (NIH publication No. 85–23, revised 1996).

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2.1. Preparation of animal experiment

In this study, we divided 9-week-old male C57BL/6J mice (23–30 g) into 4 groups as follows: (1) the mice to which MAP of 30 mg/kg was intraperitoneally administered only (Group S−M+), (2) the mice which were restrained after the administration of MAP at 30 mg/kg (Group S+M+), (3) the mice which were restrained after the intraperitoneal administration of saline (10 ml/kg) (Group S+M−), and (4) the mice to which saline was intraperitoneally administered only (Group S−M−). The mice which were restrained were inserted into 50 ml centrifuge tubes, the apices of which were cut for full ventilation (BD Falcon™, Franklin Lakes, NJ). All of the mice were killed with ketamine and xlazine overanesthesia 1 h after the administration of MAP or saline, and the heart was removed immediately after death, and soaked in RNA later (Applied Biosystems, Foster City, CA) for one night, and then stored at −80 °C for later analysis of mRNA expression.

2.2. Microarray analysis

In order to obtain a focus on the genes for mRNA expression analysis, we had microarray analysis, using OpArray Mouse V4.0, carried out on the hearts removed from the mice in the 4 groups ($n = 5$ for each group) by Filgen, Inc. (Aichi, Japan). Briefly, this method has 35,852 probes to approximately 25,000 genes and about 38,000 gene transcripts. From the results of microarray analysis, we selected the mRNA of genes, the expression of which would be determined by quantitative RT-PCR method, on the basis of the criteria that mRNA expression of the gene in the Group S+M+ was at least 3-fold more than, or one third less than, that in Group S−M−, after the change caused by the administration of MAP and restraint of the body.

2.3. Quantitative RT-PCR analysis

With the other 6 or 7 hearts in each group, mRNA was extracted using QuickGene RNA tissue kit SII (FUJIFILM, Tokyo, Japan) according to the manufacturer's instructions. The extracted mRNA was then utilized as a template, and complementary DNA (cDNA) was synthesized by using a first-strand cDNA synthesis kit (ReverTra Ace-α-, Toyobo, Osaka, Japan), according to the attached instructions.

Quantitative RT-PCR analysis was performed on the expression of the mRNA of the genes selected from the results of the microarray analysis. Briefly, primer sequences of cDNA were designed using Primer Express version 1.5 (Applied Biosystems). The mRNA levels were quantified using a fluorescence detection system (ABI PRISM 7300 sequence detection system, Applied Biosystems), in accordance with the attached instructions. The PCR mixture consisted of 0.5 μM of each primer and 5 μl of Power SYBR Green Realtime PCR Master Mix (Applied Biosystems) in a final volume of 10 μl.

2.4. Cytokine analyses

The tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 levels in serum were determined by using a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen, Carlsbad, CA) following the protocol booklets.

2.5. Statistical analysis

All analyses were performed using JMP version 5, Japanese edition (SAS Institute, Cary, NC). The measurements were expressed as means ± SE. The differences among multiple groups were determined by repeated measure two-way analysis of variance (ANOVA). A P value of less than 0.05 was considered statistically significant.

3. Result

3.1. Microarray analysis

On the basis of the criteria described above, the mRNA expression of 22 genes significantly increased (Table 1), whereas that of 14 genes significantly decreased (Table 2), in the Group S+M+ compared with the Group S−M−. Of these 36 genes selected from the results of microarray analysis, 14 genes were excluded because their function is still unclear. Thus, we performed quantitative RT-PCR analysis on the remaining 22 genes.

3.2. Quantitative RT-PCR analysis

From the quantitative RT-PCR analysis on mRNA of the 22 genes (Table 3), a significant change in mRNA expression was shown in Nfkbiz (P values: body restraint (S): 0.6153, administration of MAP (M): 0.0280, interaction of body restraint and MAP (S*M): 0.3849), Nr4a1 (S: 0.3293, M: <0.0001, S*M: 0.5566), Dusp1 (S: 0.8113, M: 0.0130, S*M: 0.3980), Rgs2 (S: 0.0159, M: 0.0226, S*M: 0.9704), Rasd1 (S: 0.0005, M: 0.0134, S*M: 0.0669) and MafF (S: 0.0037, M: 0.0234, S*M: 0.9553) by repeated measure two-way ANOVA. In detail, the mRNA expression of Nfkbiz, Nr4a1 and Dusp1 was significantly up-regulated by administration of MAP, while that of MafF, Rasd1 and Rgs2 was additively up-regulated by administration of MAP and body restraint without interaction between administration of MAP and body restraint (Fig. 1a–f). On the other hand, the expression of mRNA of Iigs1 (S: 0.5508, M: 0.0007, S*M: 0.0871) was significantly lessened by administration of MAP (Fig. 1g).

3.3. Cytokine analyses

The serum concentrations of TNF-α, IL-1β and IL-6 in each group are shown in Table 4. The serum concentration of TNF-α

Table 1
The genes in Group S+M+, the expression of which increased at least 3-fold over those in Group S−M− by microarray analysis.

Name of the genes	Function
Cd14	Mediate LPS-induced cell activation
Cfc1	Intercellular signaling pathway during vertebrate embryogenesis
Dusp1	MAP kinase phosphatase
Efcbp2	N-terminal EF-hand calcium binding protein
Gak	CyclineG associated kinase
Iigs1	IFN-inducible GTPase
MafF	Small Maf basic leucine zipper transcription factor
Nfkbiz	Regulator of NfκB
Nr4a1	Transcription factor
Olfr492	Olfactory receptor
Pold3	DNA polymerase
Rasd1	Activator of G-protein signaling
Rgs2	Regulator of G-protein signaling
Rnf125	Mediator of ubiquitin ligase activity
Zdhhc23	Zinc-finger, DHHC-type family
RIKEN cDNA 1300002F13 gene	–
(AK004796)[a]	–
(AK013890)[a]	–
(AK019206)[a]	–
(AK032137)[a]	–
(AK033622)[a]	–
(AK036770)[a]	–

[a] Riken full length clone.