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Methamphetamine enhances Hepatitis C virus replication in human hepatocytes

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SUMMARY

Very little is known about the interactions between hepatitis C virus (HCV) and methamphetamine, which is a highly abused psychostimulant and a known risk factor for human immunodeficiency virus (HIV)/HCV infection. This study examined whether methamphetamine has the ability to inhibit innate immunity in the host cells, facilitating HCV replication in human hepatocytes.

Methamphetamine inhibited intracellular interferon alpha expression in human hepatocytes, which was associated with the increase in HCV replication. In addition, methamphetamine also compromised the anti-HCV effect of recombinant interferon alpha. Further investigation of mechanism(s) responsible for the methamphetamine action revealed that methamphetamine was able to inhibit the expression of the signal transducer and activator of transcription 1, a key modulator in interferon-mediated immune and biological responses. Methamphetamine also down-regulated the expression of interferon regulatory factor-5, a crucial transcriptional factor that activates the interferon pathway. These *in vitro* findings that methamphetamine compromises interferon alpha-mediated innate immunity against HCV infection indicate that methamphetamine may have a cofactor role in the immunopathogenesis of HCV disease.

Keywords

hepatitis C virus; interferon alpha; IRF-5; methamphetamine; STAT 1

INTRODUCTION

Hepatitis C virus (HCV) is a growing public health problem worldwide, with c. 170 million individuals chronically infected with no available vaccine [1,2]. In the United States alone, the number of HCV-infected persons is nearly 4 million. There are 40 000 estimated new HCV infections reported each year in the United States [3]. About eighty percent of HCV-infected individuals become chronic carrier, which is the leading indication for liver transplantation.

HCV infection currently causes estimated 10 000-20 000 deaths annually in the United States. This number is expected to be tripled by 2020 [4,5].

One population that is particularly affected by HCV infection is the illicit drug-using population [6-8]. Drug abusers are vulnerable to HCV infection as a result of collective use of injection equipment and drug solution, as well as through sexual behaviour. Injection drug use (IDU) is the predominant mode of HCV transmission in the United States, accounting for c. 70% of acute cases and 60% to 90% of all chronic infection [9-11]. Methamphetamine (METH) is an illicit drug abused worldwide, which is a major risk factor for human immunodeficiency virus 1 (HIV-1) infection [12,13]. METH abuse was associated with higher HIV RNA loads in highly active anti-retroviral therapy (HAART)-treated subjects [14]. As HIV/HCV co-infection is found in 50-90% of HIV-infected IDUs, chronic HCV infection is expected to lead to an increasing morbidity and mortality rate among HIV-infected populations [15-17]. A substantial proportion of METH users, both with and without HIV infection has been infected with HCV [18-21], suggesting that METH abuse is also a risk factor for HCV infection. About 15% of total METH-dependent users and 44% of the injection METH users were found to be infected with HCV [22]. Therefore, METH use and HIV/HCV infections are overlapping epidemics in the United States and worldwide. Although risky behavioural practices, such as sharing contaminated needles and sexual activity after using METH may play important role in the transmission of HCV, there is little information available about whether METH itself can enhance HCV infection and promote viral replication, thus promoting HCV disease progression in HCV-infected METH abusers or increasing susceptibility to HCV infection in HCV-seronegative individuals exposed to METH. The lack of knowledge about the impact of METH on HCV disease is a barrier to the fundamental understanding of HCV infection-related morbidity and mortality among drug abusing population and to the development of new therapeutic approaches for HCV infection. In this study, we examined whether METH has the ability to promote HCV replication and impair the anti-HCV effect of interferon alpha (IFN- α) in human hepatic cells. We also investigated the molecular mechanisms involved in the effect of METH on HCV replication in human hepatic cells.

METHODS

Reagents

Methamphetamine and rabbit polyclonal antibody against actin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Recombinant human IFN- α was purchased from R & D Systems Inc. (Minneapolis, MN, USA). Enzyme-linked immunosorbent assay (ELISA) kit for IFN- α protein was purchased from PBL Biomedical Laboratories (Piscataway, NJ, USA). Rabbit polyclonal anti-HCV NS5A antibody was obtained from Chiron Company (Emeryville, CA, USA). Rabbit polyclonal antibodies against IFN regulatory factor-3 (IRF-3), IRF-7, and signal transducer and activator of transcription 1 (STAT 1) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Goat anti-IRF-5 antibody was purchased from Abcam Inc. (Cambridge, MA, USA). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG and HRP-conjugated donkey anti-goat IgG were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA). CellTiter 96® AQueous One Solution Cell Proliferation Assay kit was purchased from Promega (Madison, WI, USA).

Human hepatic cell lines and HCV plasmids

Hepatic cell line (Huh7.5.1) was kindly provided by Dr. Charles Rice (Laboratory of Virology and Infectious Diseases, Rockefeller University, NY, USA). A stable cell line Huh7-JFH1 contains a chromosomally integrated HCV genotype 2a (JFH1) cDNA and constitutively produces infectious virus [23]. Cells were maintained in complete Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 10 mM Hepes, 100 units/mL penicillin, 100

mg/mL streptomycin, and 2 mM L-glutamine at 5% CO₂. The plasmid pJFH1 that contains the full-length HCV genotype 2a (JFH1) cDNA downstream of the T7 RNA promoter was kindly provided by Dr. Takaji Wakita (Department of Microbiology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan).

Preparation of infectious HCV JFH1 and HCV infection of hepatocytes

For generating infectious HCV JFH1, *in vitro* transcribed genomic JFH1 RNA was transfected into Huh7.5.1 cells as described [24]. Cell culture media collected at day 10 post-transfection were centrifuged and passed through 0.22 µm filter. The cell-free media were used as HCV JFH1 stocks, which were aliquoted and stored at -80 °C. Infection of naïve hepatocytes with HCV JFH1 was carried out as described [24].

The cytotoxic effect of METH treatment on the hepatic cells

The METH concentrations used in this study (10^{-7} - 10^{-4} M) were based on the published study [25] and our pilot experiments. These concentrations are consistent with those in blood of METH abusers [26,27]. The impact of METH treatment on the viability of hepatic cells was demonstrated by trypan blue dye exclusion assay and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay. Huh7.5.1 cells were plated in a 24-well plate (5×10^4 cells/well, for trypan blue exclusion assay) or 96-well plate (2×10^4 cells/well, for MTS assay) and cultured for 24 h and then treated with or without METH every 24 h (10^{-7} - 10^{-4} M) for 72 h. The cells were then harvested with trypsin and stained with 0.4% trypan blue. The trypan blue-positive and negative cells were counted by a haemocytometer under microscopy. For MTS assay, 20 µL of CellTiter 96® AQueous One Solution Reagent containing MTS and phenazine ethosulphate were added to each well of 96-well plate. Absorbance at 490 nm was measured after 3 h.

Methamphetamine treatment

Huh7.5.1 cells were plated in 24-well plates (5×10^4 cells/well) and cultured for 24 h and then treated with or without METH. To determine the dose-dependent effect of METH on endogenous IFN-α expression, Huh7.5.1 cells were treated with or without METH (10^{-7} - 10^{-4} M) for 24 h. To investigate the time-course effect of METH on IFN-α expression, Huh7.5.1 cells were incubated with or without METH at 10^{-5} M for different time points (METH was only added once to the cultures). To investigate whether METH enhances HCV replication in the hepatic cells, Huh7.5.1 cells were treated with or without METH (10^{-5} M) immediately after HCV JFH1 infection. The cells were then treated with METH every 24 h. To determine whether METH interferes with the anti-HCV effect of IFN-α, JFH1-infected Huh7.5.1 cells were incubated with recombinant IFN-α (100 U/mL) and/or METH (10^{-5} M) at day 3 post-infection. The stable Huh7 cell line chromosomally integrated with JFH-1 cDNA was also treated under the same conditions as described above. The cells were then treated with METH every 24 h and HCV RNA levels were analysed at different time points (48 and 72 h) of post-METH treatment.

Real time reverse transcriptase PCR

Total RNA was extracted from Huh7.5.1 cells using Tri-Reagent (Molecular Research Center, Cincinnati, OH, USA) as described [28]. Total cellular RNA (1 µg) was subjected to reverse transcription using the reverse transcription system from Promega. The real time reverse transcriptase PCR for the quantification of HCV, IFN-α, IRF-3, IRF-5, IRF-7, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was performed with the iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) as previously described [29,30]. The amplification results were visualized and analysed using the software myiQ provided with the thermocycler (iCycler iQ real time PCR detection system, Bio-Rad).

Laboratories). The levels of GAPDH mRNA were used as an endogenous reference to normalize the quantities of target mRNA. The special oligonucleotide primers used in this study were listed as follows: HCV: 5'-RAY CACTCC CCT GTG AGG AAC-3' (sense) and 5'-TGR TGC ACG GTC TAC GAG ACC TC-3' (anti-sense). IFN- α : 5'-TTT CTC CTG CCT GAA GGA CAG-3' (sense) and 5'-GCT CAT GAT TTC TGC TCT GAC A-3' (anti-sense). IRF-3: 5'-ACC AGC CGT GGA CCA AGA G-3' (sense) and 5'-TAC CAA GGC CCT GAG GCA C-3' (anti-sense). IRF-5: 5'-AAG CCG ATC CGG CCA A-3' (sense) and 5'-GGA AGT CCC GGC TCT TGT TAA-3' (anti-sense). IRF-7: 5'-TGG TCC TGG TGA AGC TGG AA-3' (sense) and 5'-GAT GTC GTC ATA GAG GCT GTT GG-3' (anti-sense). GAPDH: 5'-GGT GGT CTC CTC TGA CTT CAA CA-3' (sense) and 5'-GTT GCT GTA GCC AAA TTC GTT GT-3' (anti-sense). The oligonucleotide primers were synthesized by Integrated DNA Technologies Inc. (Coralville, IA, USA).

Western blot

Total cell lysates from the hepatic cells were prepared using a radioimmune precipitation assay (RIPA) buffer (Promega) with 1% protease inhibitor cocktail (Sigma-Aldrich Co.). Protein concentrations were determined by detergent-compatible protein assay kit (Bio-Rad Laboratories). Western blot was carried out as previous described [28]. HCV NS5A, IRF-3, IRF-7, STAT 1, and actin proteins were detected using rabbit anti-HCV NS5A (1:2 000), anti-IRF-3 (1:1 000), anti-IRF-7 (1:1 000), anti-STAT 1(1:1 000), and anti-actin (1:3 000) polyclonal antibodies, respectively. HRP-conjugated goat anti-rabbit antibody (1:10 000) was used as the second antibody. IRF-5 proteins were detected using goat anti-IRF-5 (1:250) polyclonal antibody and HRP-conjugated donkey anti-goat antibody (1:5 000). The bound antibodies were recognized by using SuperSignal West Pico Chemiluminescent Substrate Kit (Pierce, Rockford, IL, USA) according to the manufacturer's instruction. Prestained molecular markers (Bio-Rad Laboratories) were used to determine molecular weight of immuno-reactive bands.

ELISA

Total cell lysates from the cultured hepatic cells were prepared using a RIPA buffer (Promega). ELISA assay for IFN- α was performed according to the protocol provided by the manufacturer (PBL Biomedical Laboratories).

Statistical analysis

Where appropriate, data were expressed as mean $\pm$ SD from three independent experiments. For comparison of the mean of the two groups (METH *vs* untreated control cells), statistical significance was assessed by Student's *t*-test. Calculations were performed with stata Statistical Software (StataCorp, College Station, TX, USA). Statistical significance was defined as $P < 0.05$.

RESULTS

Methamphetamine (10^{-7} - 10^{-4} M) has no cytotoxic effect on the hepatic cells

Following 72 h treatment with METH (10^{-7} - 10^{-4} M), there were no significant changes in the per cent of viable cells between METH-treated and untreated cultures as determined by trypan blue staining (Fig. 1a). In addition, quantitative analysis by MTS assay showed that METH at the highest concentration of 10^{-4} M had little effect on the cell proliferation (Fig. 1b).

Methamphetamine suppresses endogenous IFN- α expression in human hepatocytes

We first examined the effect of METH on endogenous IFN- α expression in Huh7.5.1 cells. While METH at 10^{-7} M had little effect on IFN- α mRNA and protein expression (Figs 2a,b),

significant decrease in IFN- α mRNA and protein expression was observed in Huh7.5.1 cells treated with METH at the concentrations of 10^{-4} - 10^{-6} M (Figs 2a,b). We also determined whether the effect of METH on IFN- α mRNA and protein expression is time-dependent. Optimal effect of METH on IFN- α at the levels of mRNA and protein was observed within 24 h post-treatment (Figs 3a,b).

Methamphetamine enhances HCV replication

As METH has the ability to down-regulate endogenous IFN- α expression, we hypothesized that METH may enhance HCV replication in the hepatic cells. A significant increase of HCV RNA was observed in Huh7.5.1 cells infected with HCV JFH1 and treated with METH (Fig. 4). This enhancement of HCV RNA by METH was time-dependent, as the levels of HCV RNA increased from c. 2-fold at day 3 to 12-fold at day 11 post-METH treatment (Fig. 4). The increase of HCV replication by METH was also confirmed by Western blot assay (Fig. 5), showing that HCV-infected and METH-treated hepatocytes had significantly higher levels of HCV NS5A protein than the control cells.

Methamphetamine compromises the anti-HCV activity of recombinant IFN- α

Recombinant IFN- α -based treatment is effective for people infected with HCV. We examined whether METH has a negative impact on the anti-HCV activity of recombinant IFN- α . When added to HCV JFH1-infected Huh7.5.1 cells or the stable Huh7 cells chromosomally integrated with JFH1 cDNA (Huh7-JFH1), the recombinant IFN- α significantly inhibited HCV replication (Figs 6a,b). When HCV-infected Huh7.5.1 cells were treated with recombinant IFN- α for 72 h, there was >90% decrease in HCV RNA levels (Fig. 6a). However, this anti-HCV effect of recombinant IFN- α was compromised by METH, which was observed in both JFH1-infected Huh7.5.1 cells (acute infection) (Fig. 6a) and stable Huh7 cells chromosomally integrated with JFH1 cDNA (chronic infection) (Fig. 6b). In addition, the inhibitory effect of METH on the anti-HCV activity of recombinant IFN- α was also demonstrated in the experiments showing that IFN- α had a diminished effect on suppressing HCV NS5A protein expression in the hepatocytes treated with METH (Fig. 7).

Methamphetamine suppresses STAT 1 and IRF-5 expression

We hypothesized that METH suppresses the expression of STAT 1, a key factor that regulates type I IFN expression. As expected, METH-treated Huh7.5.1 cells had lower levels of STAT 1 expression than untreated Huh7.5.1 cells (Fig. 8). IRF-3, IRF-5, and IRF-7 are three key members of the IRF family, which have a crucial role in the activation of IFN antiviral pathway [31,32]. Thus, we also examined whether METH has the ability to inhibit the expression of these three key members of the IRF family. METH treatment resulted in a significant decrease of IRF-5 mRNA expression in Huh7.5.1 cells. However, METH had little effect on the expression of IRF-3 and IRF-7 (Fig. 9a). The inhibition of IRF-5 was confirmed by the Western blot assay, showing that METH-treated hepatocytes had lower levels of IRF-5 protein than the control cells (Fig. 9b). Similar to the mRNA data, the expression of IRF-3 and IRF-7 were not affected by METH treatment (Fig. 9b).

DISCUSSION

Because METH abusers often share contaminated needles and have high risk sexual activity, they are vulnerable to HCV infection. However, we know little about whether there is an association between METH use/abuse and HCV replication in human hepatocytes. METH was reported to have immunosuppressive effect, which might promote viral infections including HCV infection. METH exposure significantly inhibits T-/B-lymphocyte proliferation, T-helper 1 cytokine [Interleukin-2 (IL-2) and IFN- γ] production in splenocytes [33,34]. METH was also able to significantly increase tumour necrosis factor- α and IL-6 expression [33] which might

have potential to enhance HIV [35]/HCV [36] replication. While there are the substantial studies investigating the impact of METH on the functions of the immune system, the role of METH in modulating intracellular innate immunity against HCV in human hepatic cells remains largely unknown.

As host innate immune response has a crucial role in determining the consequences of HCV infection, we examined the effect of METH on the innate immunity against HCV replication in human hepatocytes. We are particularly interested in IFN- α , a critical component in the host innate immunity. Given the essential function of IFN- α in mediating innate immunity against HCV, intracellular IFN- α in the hepatocytes is a vital determinant of whether HCV can persist in the cells. Thus, the suppression of intracellular IFN- α would provide a favourable microenvironment for HCV replication in the hepatic cells. Our early study showed that the endogenous IFN- α plays a vital role in controlling HCV replicon replication in human hepatocytes [30]. In this study, we use newly developed infectious HCV (JFH1) cell system to examine whether METH suppresses endogenous IFN- α expression in the hepatocytes and facilitates HCV infection and replication. Our data that the expression of endogenous IFN- α in human hepatic cells was significantly inhibited by METH provide direct *in vitro* evidence that METH has the ability to compromise IFN- α -mediated innate immunity, thus promoting HCV replication in hepatocytes.

Recombinant IFN- α remains the clinical choice of treatment for chronic HCV infection today. The current available combination therapy with IFN- α and ribavirin, however, is effective in less than 50% of treated HCV-infected individuals [37,38]. The mechanism(s) involved in resistance to IFN- α therapy remains unknown. We reasoned that METH has the ability to compromise the anti-HCV effect of IFN- α . Our study showed that recombinant IFN- α has a diminished effect on suppressing HCV replication in METH-treated hepatocytes. Although the mechanism(s) responsible for the observed METH action remain to be determined, these *in vitro* data suggested that the effectiveness of IFN- α treatment for METH abusers with HCV infection may be diminished. Further epidemiological and clinical studies are warranted to determine whether METH use contributes in part to the resistance of IFN- α -based therapy *in vivo*.

Our investigation on the mechanism(s) involved in the METH action on IFN- α expression showed that METH inhibits the expression of STAT 1 (Fig. 8). The Janus kinase-STAT signalling pathway is the major pathway for IFN-mediated signalling and activation of gene expression [39] (Fig. 10). STAT 1 plays a crucial role in mediating IFN-dependent biological responses including activation of the antiviral state and induction of type I IFNs' expression [40-42] (Fig. 10). Thus, the suppression of STAT 1 expression by METH may be a key mechanism for METH-mediated inhibition of endogenous IFN- α in hepatocytes. In addition, METH inhibits IRF-5 expression in hepatocytes at both mRNA and protein levels. This effect of METH appeared to be specific, as the expression of IRF-3 and IRF-7, two key members in the IRF family, was not affected by METH treatment (Fig. 9). The IRFs, like the STATs are important regulators in the type I IFN-mediated antiviral response (Fig. 10). These regulators not only recognize the elements of DNA promoter to modulate type I IFN genes selectively, but also regulate the IFN-stimulated response element in some IFN-stimulated genes leading to induction of an antiviral state [43,44]. Recent studies [45,46] have further demonstrated an essential role of IRF-3, IRF-5, and IRF-7 in the activation of type I IFN genes and the induction of an antiviral state. While IRF-3 and IRF-7 are both primary players in the activation of IFN- α and IFN- β , respectively, IRF-5 was also characterized as an important factor participating in the induction of IFN- α/β [43,46,47]. IRF-5 is expressed constitutively in lymphoid tissues and blood cells at low levels, but its expression is enhanced by viral infection or IFN- α/β treatment [43]. Like IRF-3 and IRF-7, IRF-5 requires phosphorylation-induced activation to translocate to the nucleus to activate IFNs. The evidence that METH-inhibited expression of IRF-5 in

hepatocytes provides an additional molecular mechanism involved in METH-mediated IFN- α inhibition, suggesting that METH affects multiple steps of the IFN- α signalling pathway in hepatic cells (Fig. 10). These findings indicate that METH-mediated alteration of the IFN signalling pathway is responsible for a compromised intracellular innate immunity, which contributes to HCV replication and persistence in the hepatic cells.

Collectively, our study for the first time provides direct *in vitro* evidence that METH suppresses endogenous IFN- α expression and promotes HCV replication in the hepatic cells. In addition, we have presented the data that METH compromises the anti-HCV activity of recombinant IFN- α . The information is important as METH-dependent users are at significant risk for HCV infection[22]. Thus, IFN- α -based treatment for METH-using population with HCV infection remains a significant challenge. As we used the only available HCV 2a strain for this study and the genotype 1 strains of HCV are the most prevalent genotypes associated with liver disease in the United States and most region of the world [48,49], future investigations using other strains of HCV are necessary to illustrate METH-mediated immunosuppressive mechanisms that contribute to *in vivo* HCV persistence in the target cells and promote HCV disease progression.

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Fig. 1.

The cytotoxic effect of methamphetamine (METH) on Huh7.5.1 cells. Huh7.5.1 cells were treated with or without METH every 24 h at indicated concentrations for 72 h. The per cent of viable cells and cell proliferation was determined by trypan blue dye exclusion assay (A) and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay (B). The results are the mean $\pm$ SD of three independent experiments.

Fig. 2.

Dose-dependent effect of methamphetamine (METH) on endogenous interferon- α (IFN- α) mRNA (A) and protein (B) expression in human hepatocytes. (A) Huh7.5.1 cells were incubated with or without METH at indicated concentrations for 24 h. Cellular RNA was subjected to the real time reverse transcriptase PCR for IFN- α mRNA. Data are expressed as IFN- α mRNA levels in Huh7.5.1 cells treated with METH relative (%) to those in the untreated control cells (defined as 100). (B) Cell lysates from Huh7.5.1 cells incubated with or without METH at indicated concentrations for 24 h were subjected to enzyme-linked immunosorbent assay for IFN- α . The results are the mean $\pm$ SD of three independent experiments (*, $P < 0.05$; **, $P < 0.01$, METH vs control).

Fig. 3.

Time-course effect of methamphetamine (METH) on endogenous interferon- α (IFN- α) mRNA (A) and protein (B) expression in human hepatocytes. (A) Huh7.5.1 cells were incubated with or without METH (once) at 10^{-5} M for indicated time points. Cellular RNA was subjected to the real time reverse transcriptase PCR for IFN- α mRNA. Data are expressed as IFN- α mRNA levels in Huh7.5.1 cells treated with METH relative (%) to those in the untreated control cells (defined as 100). (B) Cell lysates from Huh7.5.1 cells incubated with or without METH for indicated times were subjected to enzyme-linked immunosorbent assay for IFN- α protein. Data are expressed as IFN- α protein levels in Huh7.5.1 cells treated with METH relative (%) to

those in the untreated control cells (defined as 100). The results are the mean $\pm$ SD of three independent experiments (*, $P < 0.05$; **, $P < 0.01$, METH *vs* control).

Fig. 4.

Effect of methamphetamine (METH) on hepatitis C virus (HCV) replication in human hepatocytes. Huh7.5.1 cells were incubated with HCV JFH1 for 6 h and then treated with or without METH (10⁻⁵ M). METH was added to the cell cultures every 24 h. Cellular RNA was collected at indicated time points and subjected to real time reverse transcriptase PCR for HCV and glyceraldehyde-3-phosphate dehydrogenase mRNA. Data are expressed as HCV RNA levels relative (fold) to untreated control, which is defined as 1.0. The results shown are the mean $\pm$ SD of three independent experiments (*, $P < 0.05$; **, $P < 0.01$, METH vs control).

Fig. 5.

Western blot analysis of hepatitis C virus (HCV) NS5A protein expression in JFH1-infected human hepatocytes treated with or without methamphetamine (METH). HCV JFH1-infected Huh7.5.1 cells were incubated with or without METH (10^{-5} M) for 11 days. Equal amount of proteins extracted from METH-treated or untreated cells were subjected to Western blot assay using rabbit antibodies against HCV NS5A and actin. The numbers in the right panel are the signal intensities of protein bands of the Western blot expressed as densitometry scanning units. One representative of three experiments with similar results is shown.

Fig. 6.

Effect of methamphetamine (METH) on the anti-hepatitis C virus (anti-HCV) activity of recombinant interferon- α (IFN- α) in human hepatocytes. JFH1-infected Huh7.5.1 cells (acute infection) (A) and Huh7 cells that chromosomally integrated with JFH1 cDNA (chronic infection) (B) were incubated with or without IFN- α (100 U/mL) and/or METH (10^{-5} M) for 48-72 h. Cellular RNA was collected at the indicated time points and subjected to real time reverse transcriptase PCR for HCV and glyceraldehyde-3-phosphate dehydrogenase mRNA. The data are expressed as HCV RNA levels in cells treated with IFN- α or/and METH relative (%) to those in the untreated control (defined as 100). The results are the mean $\pm$ SD of three independent experiments (*, $P < 0.05$, IFN- α vs IFN- α + METH).

Fig. 7.

Western blot analysis of hepatitis C virus (HCV) NS5A protein expression in JFH1-infected human hepatocytes treated with interferon- α (IFN- α) and/or methamphetamine (METH). JFH1-infected Huh7.5.1 cells were incubated with or without IFN- α (100 U/mL) and/or METH (10^{-5} M) for 72 h. Equal amount of proteins was subjected to the Western blot assay using rabbit antibodies against HCV NS5A and actin. The numbers in the panel are the signal intensities of protein bands of the Western blot expressed as densitometry scanning units. One representative of three experiments with similar results is shown.

Fig. 8.

Effect of methamphetamine (METH) on signal transducer and activator of transcription (STAT 1) expression in human hepatocytes. Huh7.5.1 cells were incubated with or without METH (10^{-5} M) for 24 h. Equal amount of proteins extracted from the cells was subjected to Western blot assay using rabbit antibodies against STAT 1 and actin. The numbers in the right panel are the signal intensities of protein bands of the Western blot expressed as densitometry scanning units. One representative of three experiments with similar results is shown.

Fig. 9.

Effect of methamphetamine (METH) on the expression of IFN regulatory factors (IRFs) in human hepatocytes. (A) Real time reverse transcriptase PCR analysis of IRFs mRNA expression. Huh7.5.1 cells were incubated with or without METH (10⁻⁵ M) for the indicated time points. Cellular RNA was collected and subjected to real time RT PCR for IRF-3, IRF-5, IRF-7 and glyceraldehyde-3-phosphate dehydrogenase mRNA. The data are expressed as IRF mRNA levels relative (%) to untreated control, which is defined as 100. The results shown are the mean $\pm$ SD of three independent experiments (**, $P < 0.01$, METH *vs* control). (B) Western blot analysis of IRF proteins expression. Huh7.5.1 cells were incubated with or without METH (10⁻⁵ M) for 24 h. Equal amount of proteins was subjected to Western blot assay using antibodies against IRF-3, IRF-5, IRF-7 and actin. The numbers in the right panel are the signal intensities of protein bands of the Western blot expressed as densitometry scanning units. One representative of three experiments with similar results is shown.

Fig. 10.

Schematic diagram of the mechanisms involved in methamphetamine (METH)-mediated suppression of intracellular interferon- α (IFN- α) expression. In addition to its inhibiting effect on signal transducer and activator of transcription (STAT 1), METH in conjunction with hepatitis C virus (HCV) proteins (NS5A and NS3/4A) inhibit the expression of the key IFN regulatory factors (IRFs), resulting in the down-regulation of IFN- α . Abbreviation: IRF, interferon regulatory factor; STAT, signal transducer and activator of transcription; ISGF3, IFN-stimulated gene factor-3; PKR, protein kinase; OAS, oligoadenylate synthetase; Mx, myxovirus-resistance proteins; JAK, Janus-activated kinase; TYK, tyrosine kinase.