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The Effect of Methamphetamine on the mRNA Level for 14·3·3 η Chain in the Human Cultured Cells

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Abstract

14·3·3 protein, a brain-specific protein, is an activator of tyrosine and tryptophan hydroxylases, key enzymes for biosynthesis of dopamine and serotonin. In this article, we describe cloning of cDNA for human brain 14·3·3 η chain and expression of 14·3·3 η chain mRNA in some human cultured cells. The cloned cDNA is 1730 bp long and contains 191 bp of a 5'-noncoding region, the complete 738 bp of coding region, and 801 bp of a 3'-noncoding region, containing three polyadenylation signals. This cDNA encoded a polypeptide of 246 amino acids (M_r 28,196). Furthermore, using *in situ* hybridization histochemistry, the expression of mRNA for this protein was examined in the rat central nervous system. *In situ* hybridization histochemistry indicated that 14·3·3 η chain mRNA is detected not only in the monoamine-synthetic neurons, but also in other neurons in the discrete nuclei, which synthesize neither cathecholamine nor serotonin. Northern blot analysis demonstrated that the addition of methamphetamine into the cultured medium increased the mRNA level for 14·3·3 η chain in U-251 cells, but did not increase that of GFAP.

Index Entries: 14·3·3 protein; human cell lines; cDNA; mRNA; methamphetamine; *in situ* hybridization.

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Introduction

14-3-3 Protein is one of the brain-specific proteins that was discovered by Moore and Perez (1967) about 30 years ago. Recently, we found that 14-3-3 protein activates tyrosine hydroxylase and tryptophan hydroxylase in the presence of Ca^{2+}/calmodulin-dependent kinase II (Ichimura et al., 1987). Both hydroxylases are rate-limiting key enzymes in the biosynthetic pathway of dopamine and serotonin, which are important considering physiological and neuropsychiatric aspects. We analyzed bovine brain 14-3-3 protein by using high-performance liquid chromatography (HPLC) and electrophoresis, and demonstrated that this protein consists of at least seven polypeptides (α, β, γ, δ, ε, ζ, and η) (Ichimura et al., 1987, 1988; Isobe et al., 1991). These seven polypeptides possess the kinase-dependent activating effect on tyrosine and tryptophan hydroxylases activity.

Considering the importance of this 14-3-3 protein, we cloned cDNA for bovine and rat brain 14-3-3 protein η chains and determined the nucleotide sequences of these cDNAs (Ichimura et al., 1987; Watanabe et al., 1991).

On the other hand, methamphetamine is an interesting amine, since the repeated administration of this drug into a human may produce a schizophrenia-like addiction state. Previously, we reported the inhibition of brain hexokinase activity in the rats, when methamphetamine was repeatedly injected (Takahashi and Akabane, 1960). However, the molecular mechanism for methamphetamine addiction has not yet been clarified. In this article, we describe cloning and the nucleotide sequence of cDNA for human brain 14-3-3 protein η chain and the effect of methamphetamine on 14-3-3 mRNA level of human cultured cells in order to study the molecular mechanisms for methamphetamine addiction.

Materials and Methods

cDNA Cloning

cDNA was synthesized from poly $(A)^+$ RNA of human cerebral cortex by the method of Gubler and Hoffman (1983). After ligation to *Eco*RI-*Not*I adaptors (Pharmacia, Uppsala, Sweden), the double-stranded cDNA was ligated to the *Eco*RI-digested λgt10 arm. Following in vitro packaging, recombinant phages containing human brain 14-3-3 cDNA were screened by plaque hybridization (Benton and Davis, 1977) using the cloned bovine brain 14-3-3 cDNA as a probe. The isolated phage clones were subcloned into pUC118, and their nucleotide sequences were determined by dideoxy chain termination (Sanger et al., 1977) and chemical modification (Maxam and Gilbert, 1980).

Cell Culture

Human cultured nerve cell lines containing an astroglioma cell line, U-251 (Ponten and Westermark, 1978), and an oligodendroglioma cell line, KG-1-C (Miyake, 1979), were used in this study. In the case of methamphetamine experiments, methamphetamine (Dainippon Pharmaceutical Corp., Osaka, Japan) was added into the cultured medium containing the cells at final concentrations of 10^{-4}, 10^{-5}, 10^{-6}, and $10^{-7}M$.

RNA Extraction and Northern Blot Hybridization

Total RNA was extracted from adult human cerebral cortex and several cultured cells by the guanidium thiocyanate/CsCl procedure (Chirgwin et al., 1979), and poly $(A)^+$ RNA was purified from total RNA by oligo(dT)cellulose chromatography (Aviv and Leder, 1972). About 10 μg of total RNA were used for formamide gel electrophoresis and Northern blot analysis according to the method of Thomas (1980). The DNA fragments from the bovine cDNA or the human brain cDNA were used for hybridization as a probe.

In Situ *Hybridization Histochemistry*

Fresh frozen sections of rat brain (4–5 wk old) were prepared by cryostat, mounted on gelatin-coated slide glasses, and stored at –80°C until use. Slides were dipped in 4% paraform-

aldehyde–0.1*M* sodium phosphate buffer (pH 7.2) for 20 min and 2 mg/mL of glycine in phosphate-buffered saline for 20 min, and subsequently acetylated in 0.25% acetate in 0.1*M* Tris-HCl (pH 8.0). Hybridization was carried out at 37°C overnight with a [^{35}S]dCTP-labeled probe at a concentration of 5×10^5 dpm/50 μL of hybridization solution consisting of 4X SSC, 50% formamide, 1X Denhardt's solution, 10% dextran sulfate, 0.1*M* sodium phosphate buffer (pH 7.2), 2% sarkosyl, 0.1*M* dithiothreitol, and 250 μg/mL of heat denatured salmon sperm DNA. The slides were then washed three times in 0.1X SSC–0.1% sarkosyl at 37°C for 40 min, and autoradiographed using NTB2 nuclear track emulsion (Kodak) for 3 wk.

Results

cDNA Cloning and the Nucleotide Sequence of cDNA for Human 14·3·3 Protein η Chain

We isolated a cDNA clone for the human 14·3·3 protein η chain mRNA by screening human brain cDNA library using the bovine cDNA as a probe. For sequence determination, the longest cDNA clone (1730 bp) was subcloned into pUC118 plasmid.

Figure 1 shows the nucleotide sequence and deduced amino acid sequence of human 14·3·3 η chain cDNA. This cDNA is 1730-bp long and contains 738 bp of the coding region encoding a polypeptide of 246 amino acids (M_r 28,196), 191 bp of 5'-noncoding region with G-rich sequence, and 801 bp of 3'-noncoding region with three polyadenylation signals (AATAA). The nucleotide and deduced amino acid sequences show that they have high homology among bovine, rat, and human. Only two amino acids of human η chain at positions 102 and 164 are different from those of bovine η polypeptide.

Northern Blot Analysis of 14·3·3 Protein η Chain mRNA

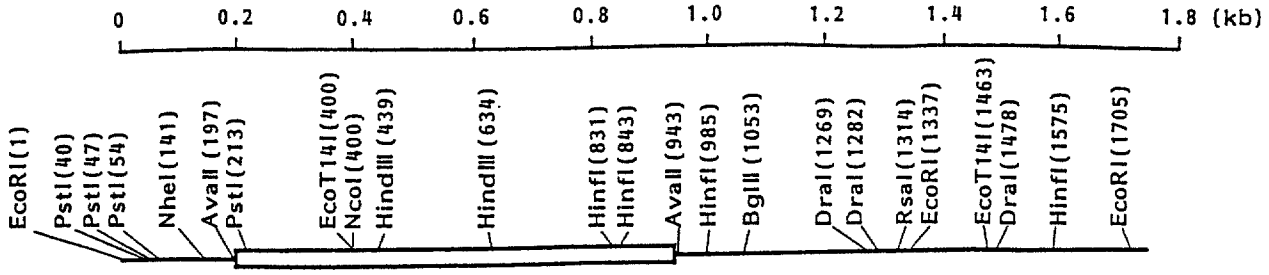
Figure 2 shows Northern blot analysis of 14·3·3 protein η chain mRNA in human cerebral cortex and two cultured nerve cell lines, including U-251 (an astroglioma cell line) and KG-1-C (an oligodendroglioma cell line). We could detect 1.8-kb mRNA bands using the cDNA fragment as a probe. The level of mRNA in the cultured cell lines is lower than that of human cerebral cortex, although some tailing bands were found in the low-mol-wt regions.

In Situ *Hybridization Histochemistry of 14·3·3 Protein η Chain mRNA*

In addition to the Northern blot analysis, *in situ* hybridization histochemistry was carried out to study the cellular distribution of 14·3·3 protein η chain mRNA in the nervous tissue. However, since we could not obtain fresh human brain tissue for this study, we had to use the rat brain for this purpose. Figure 3 shows a coronal section through the midbrain of rat. An intensive signal of 14·3·3 mRNA expression was found in the magnocellular portion of the red nucleus and the oculomotor nucleus. The substantia nigra showed a moderate level of the mRNA expression. A parasagittal section of cerebellum is shown in Fig. 4, indicating a distinct expression of the 14·3·3 η chain mRNA in the Purkinje cells. The cerebellar nucleus was also intensely expressed this mRNA. These observations indicate the expression of this mRNA in other types of neurons with large somata in addition to the presence of 14·3·3 η chain mRNA in tyrosine hydroxylase-containing catecholamine neurons.

Effect of Methamphetamine on the 14·3·3 Protein η Chain mRNA in U-251 Cells

In this experiment, we examined the effects of methamphetamine on the level of mRNA in U-251 cells. Methamphetamine was added into the cultured medium at the concentration of 10^{-4}, 10^{-5}, 10^{-6}, and $10^{-7}M$ for 72 h. After 72 h of cell culture, total RNA was extracted from the cultured cells, and 14·3·3 η chain mRNA level was examined by Northern blot analysis using 14·3·3 η chain cDNA as a probe. The addition of methamphetamine increased mRNA level at $10^{-6}M$. However, we could not find this change

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1                                                    5'---GAATTCGCGGCGCCGAGAGGGCGCGAGCGGCGGCGCTGCCTGCAGCCTGCAGCCTGCAGCCTCCGGCCGGCC

73 GGCGAGCCAGTGCGCGTGCGCGGCGGCGGCCTCCGCAGCGACCGGGGAGCGGACTGACCGGCGGGAGGGCTAGCGAGCCAGCGGTGTGAGGCGCGAGGCGAGGCCGAGCCGCGAGCGAC

192 ATG GGG GAC CGG GAG CAG CTG CTG CAG CGG GCG CGG CTG GCC GAG CAG GCG GAG CGC TAC GAC GAC ATG GCC TCC GCT ATG AAG GCG GTG
  1 Met Gly Asp Arg Glu Gln Leu Leu Gln Arg Ala Arg Leu Ala Glu Gln Ala Glu Arg Tyr Asp Asp Met Ala Ser Ala Met Lys Ala Val

282 ACA GAG CTG AAT GAA CCT CTC TCC AAT GAA GAT CGA AAT CTC CTC TCT GTG GCC TAC AAG AAT GTG GTT GGT GCC AGG CGA TCT TCC TGG
 31 Thr Glu Leu Asn Glu Pro Leu Ser Asn Glu Asp Arg Asn Leu Leu Ser Val Ala Tyr Lys Asn Val Val Gly Ala Arg Arg Ser Ser Trp

372 AGG GTC ATT AGC AGC ATT GAG CAG AAA ACC ATG GCT GAT GGA AAC GAA AAG AAA TTG GAG AAA GTT AAA GCT TAC CGG GAG AAG ATT GAG
 61 Arg Val Ile Ser Ser Ile Glu Gln Lys Thr Met Ala Asp Gly Asn Glu Lys Lys Leu Glu Lys Val Lys Ala Tyr Arg Glu Lys Ile Glu

462 AAG GAG CTG GAG ACA GTT TGC AAT GAT GTC CTG TCT CTG CTT GAC AAG TTC CTG ATC AAG AAC TGC AAT GAT TTC CAG TAT GAG AGC AAG
 91 Lys Glu Leu Glu Thr Val Cys Asn Asp Val Leu Ser Leu Leu Asp Lys Phe Leu Ile Lys Asn Cys Asn Asp Phe Gln Tyr Glu Ser Lys

552 GTG TTT TAC CTG AAA ATG AAG GGT GAT TAC TAC CGC TAC TTA GCA GAG GTC GCT TCT GGG GAG AAG AAA AAC AGT GTG GTC GAA GCT TCT
121 Val Phe Tyr Leu Lys Met Lys Gly Asp Tyr Tyr Arg Tyr Leu Ala Glu Val Ala Ser Gly Glu Lys Lys Asn Ser Val Val Glu Ala Ser

642 GAA GCT GCC TAC AAG GAA GCC TTT GAA ATC AGC AAA GAG CAG ATG CAA CCC ACG CAT CCC ATC CGG CTG GGC CTG GCC CTC AAC TTC TCC
151 Glu Ala Ala Tyr Lys Glu Ala Phe Glu Ile Ser Lys Glu Gln Met Gln Pro Thr His Pro Ile Arg Leu Gly Leu Ala Leu Asn Phe Ser

732 GTG TTC TAC TAT GAG ATC CAG AAT GCA CCT GAG CAA GCC TGC CTC TTA GCC AAA CAA GCC TTC GAT GAT GCC ATA GCT GAG CTG GAC ACA
181 Val Phe Tyr Tyr Glu Ile Gln Asn Ala Pro Glu Gln Ala Cys Leu Leu Ala Lys Gln Ala Phe Asp Asp Ala Ile Ala Glu Leu Asp Thr

822 CTA AAC GAG GAT TCC TAT AAG GAC TCC ACG CTG ATC ATG CAG TTG CTG CGA GAC AAC CTC ACC CTC TGG ACG AGC GAC CAG CAG GAT GAA
211 Leu Asn Glu Asp Ser Tyr Lys Asp Ser Thr Leu Ile Met Gln Leu Leu Arg Asp Asn Leu Thr Leu Trp Thr Ser Asp Gln Gln Asp Glu

912 GAA GCA GGA GAA GGC AAC TGA AGATCCTTCAGGTCCCCTAGCCCTTCCTTCACCCACCACCCCCATCATCACCGATTCTTCCTTGCCACAATCACTAAATATCTAGTGCTAA
241 Glu Ala Gly Glu Gly Asn

1024 ACCTATCTGTATTGGCAGCACAGCTACTCAGATCTGCACTCCTGTCTCTTGGGAAGCAGTTTCAGATAAATCATGGGCATTGCTGGACTGATGGTTGCTTTGAGCCCACAGGAGCTCCC
1143 TTTTTGAATTGTGTGGAGAAGTGTGTTCTGATGAGGCATTTTACTATGCCTGTTGATCTATGGGAAATCTAGGCGAAAGTAATGGGGAAGATTAGAAAGAATTAGCCAACCAGGCTACA
1262 GTTGATATTTAAAAGATCCATTTAAAACAAGCTGATAGTGTTTCGTTAAGCAGTACATCTTGTGCATGCAAAAATGAATTCACCCCTCCCACCTCTTTCTTCAATTAATGGAAAACTGT
1381 TAAGGGAAGCTGATACAGAGAGACAACTTGCTCCTTTCCATCAGCTTTATAATAAACTGTTTAACGTGAGGTTTCAGTAGCTCCTTGGTTTTGCCTCTTTAAATTATGACGTGCACAAA
1500 CCTTCTTTTCAATGCAATGCATCTGAAAGTTTTGATACTTGTAACTTTTTTTTTTTTTTGGTTGCAATTGTTTAAGAATCATGGATTTATTTTTTGTAACTCTTTGGCTATTGTCCTTG
1619 TGTATCCTGACAGCGCCATGTGTGTCAGCCCATGTCAATCAAGATGGGTGATTATGAAATGCCAGACTTCTAAAATAAATGTTTTGGAATTCAATGGGTAAATAAATGCGAC  --3'
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Fig. 1. Restriction endonuclease map and nucleotide sequence of human 14·3·3 protein η chain cDNA and the deduced amino acid sequence. The open box shows the protein-coding region. The numbers in parentheses show the positions of restriction sites. The nucleotides are numbered at the left of each line, beginning with the first nucleotide of the cDNA. The deduced 246 amino acid sequence is given below the corresponding nucleotide sequence. The number of amino acids begins with the initial Met codon. Amino acids that differ from bovine are boxed. Polyadenylation signals are underlined.

at 10^{-4}, 10^{-5}, and $10^{-7}M$. This result was reproducible. We used a densitometric scanner to analyze the quantitative differences among the data of each lane and could find a significant increase of 14·3·3 η chain mRNA in lane $10^{-6}M$, compared with those in other lanes, although there was the localized background in lane $10^{-6}M$ area (Fig. 5).

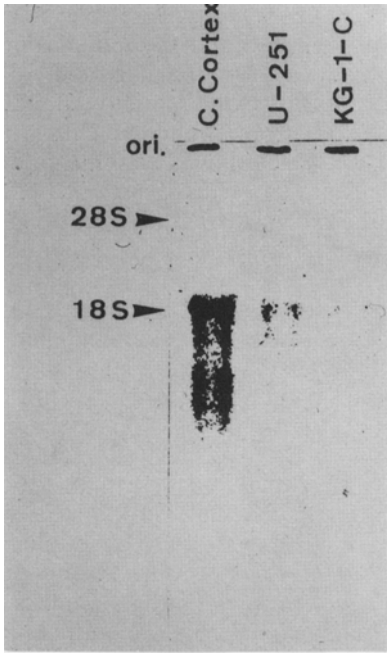

Fig. 2. Northern blot analysis. Total RNAs (10 μg) for human cerebral cortex and cell lines were hybridized with 14·3·3 cDNA fragment. The lanes show RNAs from human cerebral cortex, U-251, and KG-1-C. Ori shows origin.

Effect of Methamphetamine on the Glial Fibrillary Acidic Protein (GFAP) mRNA in the U-251 Cells

We have examined the effect of methamphetamine on the level of GFAP mRNA, which is abundant in the U-251 cells. However, mRNA levels for GFAP mRNA in the U-251 cells were not stimulated by the addition of methamphetamine at the concentration of 10^{-4}, 10^{-5}, 10^{-6}, and $10^{-7}M$ for 72 h (Fig. 6).

Discussion

We succeeded in cloning cDNA for the human 14·3·3 protein η chain and determined its nucleotide sequence. A comparison of the human and bovine 14·3·3 η chain cDNA sequences revealed 90% over the coding region, 71% in the 5'-noncoding, and 84% identity in the 3'-noncoding regions. Therefore, the amino acid sequences of the 14·3·3 proteins are highly conserved among animal species, and those of the η chain are completely identical in rat and bovine. Only two amino acids of human η chain are different from those of bovine and rat η polypeptides (Ichimura et al., 1988; Watanabe et al., 1991). Such a conservative characteristic may suggest the biological importance of this protein.

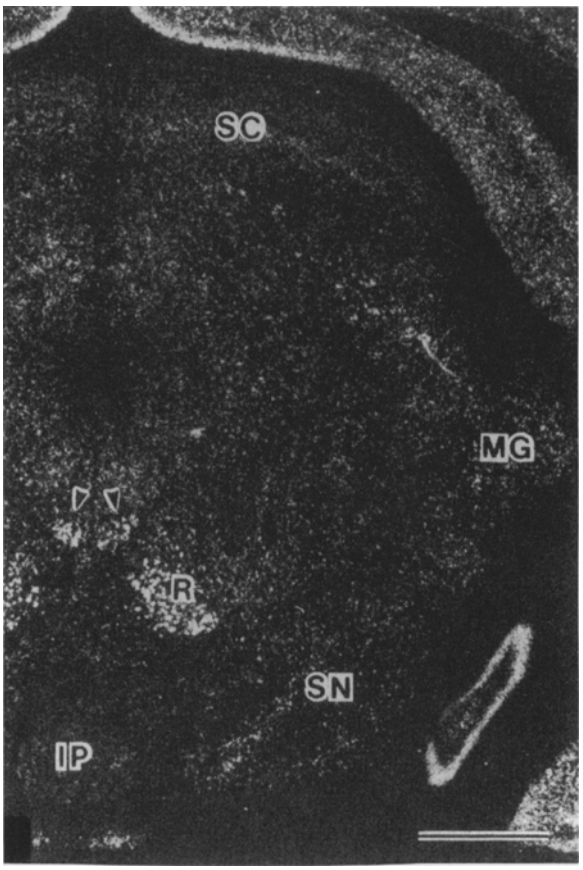

Fig. 3. *In situ* hybridization histochemistry of the rat 14·3·3 h chain mRNA (a coronal section through the midbrain). R, red nucleus; SN, substantia nigra; IP, interpedincular nucleus; MG, medial geniculate nucleus; SC, superior colliculus; arrowheads, oculomotor nucleus. Bar = 1 mm.

Northern blot analysis and *in situ* hybridization showed the presence of 14·3·3 protein η

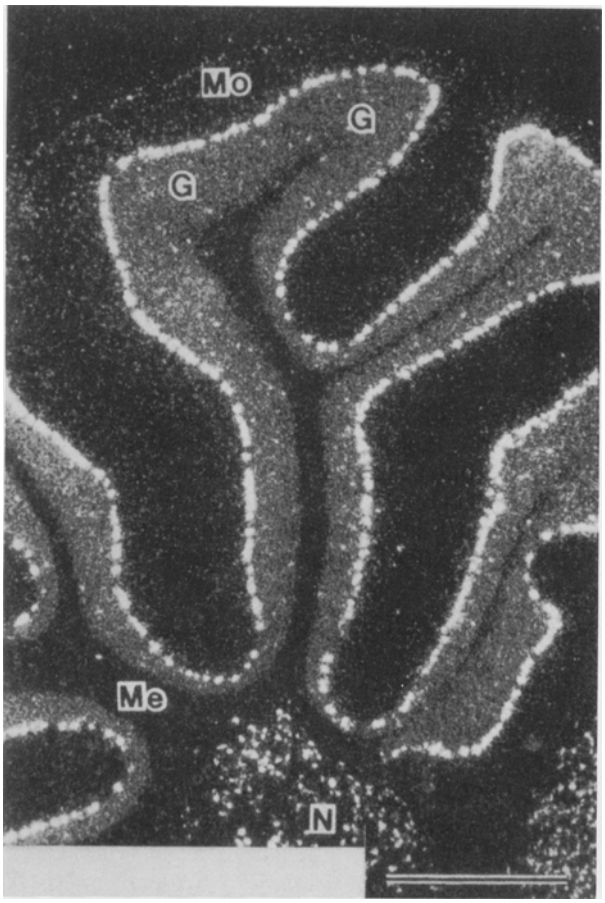

Fig. 4. *In situ* hybridization histochemistry of the rat 14·3·3 η chain mRNA (a parasagittal section of cerebellum). The Purkinje cells are located between molecular (Mo) and granular layers (G). N shows deep cerebellar nucleus. Me, medullary zone. Bar = 1 mm.

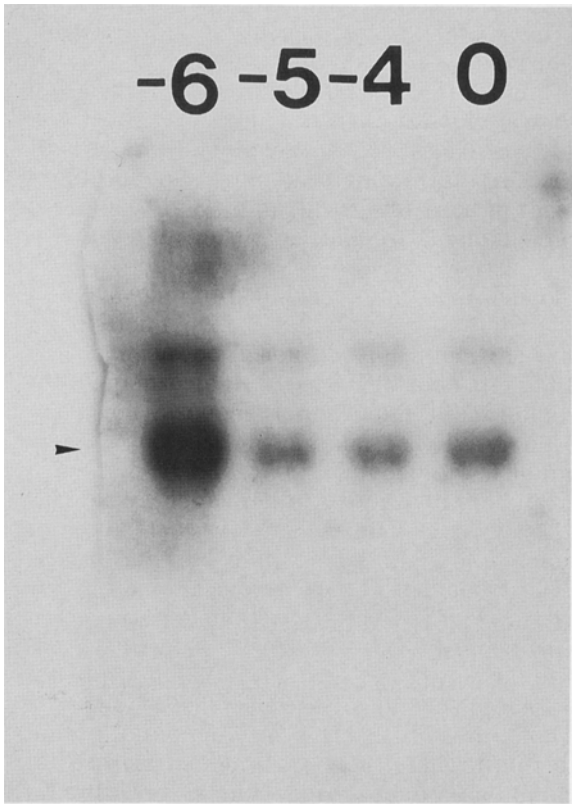

Fig. 5. The effect of methamphetamine on the mRNA level for 14·3·3 protein η chain in the U-251 cells. Total RNAs (10 μg) from U-251 cells of each lane were hybridized with 14·3·3 η chain cDNA fragment. 0, –4, –5, and –6 indicate the concentration of methamphetamine (0, 10^{-4}, 10^{-5}, and $10^{-6}M$).

chain mRNA not only in the substantia nigra and raphe nucleus, but in the cerebral cortex, cerebellum, and several cultured cell lines. This localization may suggest some other functions of this 14·3·3 protein in addition to the activating effect on tyrosine and tryptophan hydroxylases. In fact, endogenous inhibitor of protein kinase C isolated from sheep brain showed a high sequence homology to the 14·3·3 protein (Toker et al., 1990). Furthermore, Isobe et al. (personal communication) observed the activating effect of 14·3·3 protein on the protein kinase C activity. A recent paper reported that after treatment with an activator of protein kinase C (12-*O*-tetradecanoylphorbor 13-acetate or sn-1,2-diacylglycerol), the tyrosine hydroxylase mRNA level in cultured hypothalamic cells increased (Kedzierski et al., 1994). These data suggest a diverse mechanism for the activating effect of 14·3·3 protein on tyrosine hydroxylase activity.

As far as we analyzed, U-251 cells contained higher 14·3·3 η chain mRNA than other cultured cells. Therefore, we used U-251 cells for the methamphetamine experiments. We found the stimulating effect of methamphetamine on the 14·3·3 mRNA level at $10^{-6}M$ in the U-251 cells. However, we could not find such

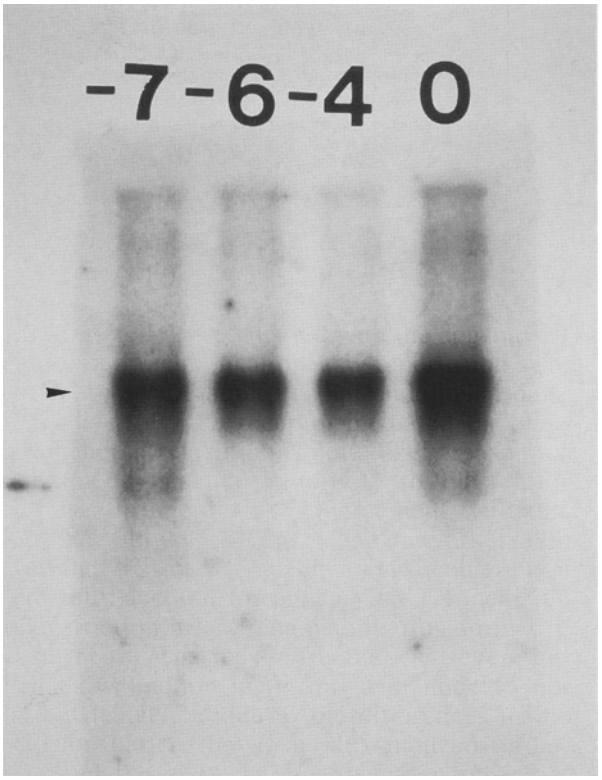

Fig. 6. The effect of methamphetamine on the mRNA level for GFAP protein in the U-251 cells. Total RNAs (10 μg) from U-251 cells of each lane were hybridized with GFAP cDNA fragment. The concentration of methamphetamine is shown as in Fig. 5.

a stimulating effect at higher concentrations of the drug. The reason for this result was not clarified. As a next step, we have to examine the time-course of the effect of methamphetamine, the effects of methamphetamine on the levels of other 14-3-3 chain mRNAs, the effects of other psychotropic drugs on the level of 14-3-3 η chain mRNA in the cultured cells, the establishment of a dose–response curve, and the use of other cell lines, such as PC12 cells.

Furthermore, in order to examine the mechanisms for the stimulating effect of methamphetamine on the level of 14-3-3 η chain mRNA in the U-251 cells, we cloned the genomic DNA for human 14-3-3 protein η chain and are analyzing the exon–intron organization, the transcription initiation site, and the nucleotide sequence of the 5'-flanking region of this gene. These studies may reveal the presence of some methamphetamine-responsive element in the 5'-flanking region.

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