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Effects of interferon-α, interferon-γ and cAMP on the transcriptional regulation of the serotonin transporter

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Abstract

We examined the effects of interferon-α and -γ, which are known to have psychiatric side effects including depression, on the transcriptional regulation of the serotonin transporter and the uptake activity of the serotonin transporter in order to clarify the involvement of the serotonin transporter in the pathogenesis of interferon-induced depression. In human placental choriocarcinoma cells (BeWo cells), both messenger RNA (mRNA) for the serotonin transporter and the imipramine-sensitive uptake of serotonin were detected. The levels of serotonin transporter mRNA were increased by treatment with interferon-α and -γ for 3 h. The increase in serotonin transporter mRNA elicited by the interferons was inhibited by treatment with actinomycin D, an inhibitor of transcription. Treatment with interferon-α or -γ for 3–6 h, but not for 30 min, increased the uptake activity of the serotonin transporter. Treatment with dibutyryl cAMP (Dib-cAMP) which was reported to up-regulate the transcription of the serotonin transporter, also increased the mRNA levels and the activity of serotonin transporter in BeWo cells. The levels of serotonin transporter mRNA gradually increased after treatment with Dib-cAMP over 24 h, while the maximal increase in serotonin transporter mRNA elicited by the interferons was detected 3 h after the treatment. The level of serotonin transporter mRNA was increased both in the midbrain and adrenal glands of mice which were treated with interferons for 3 h. These results suggest that the interferon-induced psychiatric side effects arise through regulation of serotonin transporter transcription and that the transcriptional regulation of the serotonin transporter is a possible neurochemical mechanism of affective disorders.

Keywords: 5-HT (5-hydroxytryptamine, serotonin) transporter; Interferon; Transcription; Depression; mRNA

1. Introduction

Synaptic transmission in the central nervous system is terminated by the removal of neurotransmitters, including serotonin, from the synaptic cleft by transporters present in presynaptic terminals or surrounding glial cells (Kuhar et al., 1972). The serotonin transporter plays a critical role in the termination of serotonergic neurotransmission by Na^+/Cl^--dependent uptake of serotonin into the presynaptic neuron (Blakely et al., 1991; Hoffman et al., 1991; Blakely et al., 1994; Lesch and Bengel, 1995) and also plays an important role in the uptake of extraneuronal serotonin (Carlsson et al., 1969). The serotonin transporter is also known to be a main target of antidepressants (Blakely et al., 1991; Hoffman et al., 1991) and to be involved in the pathogenesis of depression (Stanley et al., 1982). Recently, polymorphisms in the serotonin transporter gene have been reported to be associated with neuroticism (Lesch et al., 1996) and with depression (Ogilvie et al., 1996). Lesch et al. (1996) reported that a 44-bp insertion/deletion in the serotonin transporter-linked promoter region modulates the transcription of the serotonin transporter. There are a considerable number of reports of the regulation of serotonin transporter activity by various signaling pathways including cAMP (Cool et al., 1991), protein kinase C (Myers et al., 1989; Anderson and Horne, 1992), Ca^{2+} (Nishio et al., 1995), cGMP (Launay et al., 1994; Miller and Hoffman, 1994), calmodulin (Jayanthi et al., 1994) and oxidants (Bosin, 1989). It is also reported that the transcription of the serotonin transporter is regulated by interleukin-1β (Ramamoorthy et al.,

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1995b) and epidermal growth factor (Kekuda et al., 1997), suggesting that various factors such as cytokines have effects on the transcriptional regulation of the serotonin transporter, although the association between this regulation and psychiatric disorders is unknown.

Interferon comprises a family of multifunctional polypeptides which displays antiviral, antiproliferative and immunoregulatory activities (Pestka et al., 1987; Sen and Ransohoff, 1993). It is known that the interferon-receptor interaction at the cell surface leads to the activation of kinases of the Janus kinase family that then phosphorylate substrate proteins called STATs (signal transducers and activators of transcription). The phosphorylated STAT proteins then modulate gene transcription by binding specific DNA elements (Darnell et al., 1994). In recent years, the availability of human interferons of high purity and potency has enabled clinical trials of interferon therapy for acute and chronic viral illnesses, malignant diseases, and immunologic deficiency (Gutterman et al., 1982). However, the long-term administration of interferons is known to cause various side effects, especially serious psychiatric complications such as depression and suicidal ideation (Renault et al., 1987). It has also been reported that the psychiatric symptoms caused by interferon therapy are generally improved by decreasing the dose of interferon-α, and that these effects disappear after cessation of therapy (Renault et al., 1987). Furthermore, the psychiatric side effects can be treated with fluoxetine, a serotonin specific reuptake inhibitor, suggesting that the serotonin transporter is associated with the interferon-induced psychiatric complications.

In the present study, we investigated the effects of interferon-α and -γ as well as cAMP on serotonin uptake and on the transcriptional regulation of the serotonin transporter in BeWo cells, a human choriocarcinoma cell line which constitutively expresses the serotonin transporter (Jayanthi et al., 1994) and in mice.

2. Materials and methods

2.1. Materials

[^{3}H]serotonin (370 GBq mmol^{-1}) and [α-^{32}P]dCTP (3000 Ci mmol^{-1}) were purchased from Amersham (Buckinghamshire, England). Interferon-α was kindly donated by Sumitomo Pharmaceutical (Osaka, Japan) and interferon-γ was from Shionogi (Osaka, Japan). Dibutyryl cAMP (Dib-cAMP) and actinomycin D were purchased from Sigma (St. Louis, MO, USA). Murine interferon-α and -γ were purchased from Immugenex (Los Angeles, CA, USA). All other chemicals were of analytical grade. Male BALB/c Cr mice (30 ± 5 g) were housed in a temperature-controlled (24 ± 1°C) room with a 12-h light–dark cycle and given free access to food and water.

2.2. Isolation of rat serotonin transporter cDNA

To obtain rat serotonin transporter cDNA, we first performed the reverse transcriptase polymerase chain reaction (RT-PCR) for rat adrenal total RNA with primers designed according to the previously reported sequences of rat serotonin transporter cDNA (Blakely et al., 1991; Hoffman et al., 1991). The sense and antisense primers used were 5′-ACTGACCAGCAGCATGGAGA-3′ and 5′-GCTGTCACCCACACCACCTT-3′, respectively. We screened a rat brain cDNA library using an obtained polymerase chain reaction product as a probe, as previously described (Osawa et al., 1994), and isolated a cDNA encoding the full length of the rat serotonin transporter cDNA.

2.3. Cell culture and transfection

BeWo and HeLa cells were purchased from Riken Cell Bank (Tsukuba, Japan). BeWo cells were cultured in F-12 nutrient mixture with 15% fetal bovine serum. They were seeded in culture dishes 10 cm in diameter for Northern blot analysis (5×10^6 cells per dish) and in 12-well dishes (2 cm in diameter) for the [^{3}H]serotonin uptake assay. HeLa cells were cultured in minimum essential medium (MEM) with 10% fetal bovine serum. All media were supplemented with penicillin (100 units ml^{-1}) and streptomycin (100 μg ml^{-1}). For transfection, approximately 32 μg of serotonin transporter cDNA subcloned into pRC/CMV plasmid (Invitorogen, The Netherlands) was transfected into 6×10^6 HeLa cells by electroporation, using a gene pulser (Bio-Rad; 960 μF, 220 V). Transfected-cells were plated into 24-well dishes (1 cm in diameter). The [^{3}H]serotonin uptake assay was carried out 48 h after transfection as described below.

2.4. Isolation of total RNA and Northern blot analysis

After treatment with the drugs tested, total RNA was extracted, using RNeasy Mini Kit (QIAGEN, Germany), from BeWo cells that had been cultured to confluence in dishes 10 cm in diameter. Total RNA from drug-treated mice was also prepared by RNeasy Mini Kit from 50 mg of midbrain or adrenal glands. Messenger RNA (mRNA) was purified on an oligo (dT) cellulose affinity column (Oligotex-dT30 Super, TaKaRa, Japan). For electrophoresis, total RNA or mRNA was dried by vacuum centrifugation and diluted with 20 μl of loading buffer. Samples were denatured at 65°C for 5 min and loaded onto a 1.0% agarose/2.0% (0.66 M) formaldehyde/$1 \times$ MOPS (morpholinopropanesulfonic acid) gel. All the total RNA from a 10-cm dish was applied to a single lane. After electrophoresis, samples were transferred to nylon membranes (Hybond N, Amersham). The membranes were hybridized in Rapid-hyb buffer (Amersham) at 65°C for 2 h with a

^{32}P-labeled rat serotonin transporter cDNA probe, which was prepared with [α-^{32}P]dCTP by using the Random Primer DNA Labeling Kit Ver. 2 (TaKaRa). The membranes were washed twice at room temperature with 2 × SSPE/0.1% SDS (SSPE; 0.15 M NaCl, 0.01 M NaH_2PO_4, 1 mM EDTA, SDS: sodium dodecyl sulfate) for 20 min, twice at 65°C with 1 × SSPE/0.1% SDS for 15 min and twice at 65°C with 0.5 × SSPE/0.1% SDS for 15 min. The amount of mRNA was quantitated by measuring the intensity of phosphostimulated luminescence with a Bio-Imaging Analyzer (Fuji, Japan) after exposure to X-ray film. Then the membranes were subsequently stripped in boiled water and rehybridized with a ^{32}P-labeled human β-actin cDNA as an internal control for RNA loading and transfer efficiency.

2.5. [^{3}H]serotonin uptake assays

For the [^{3}H]serotonin uptake assay, BeWo cells were seeded in 12-well dishes 2 cm in diameter under sub-confluent conditions for 24 h before the assay (2×10^4 cells per well) (Nakashita et al., 1997). The culture medium was removed and replaced by Krebs–Ringer–HEPES (KRH) buffer (120 mM NaCl, 4.7 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM HEPES and 10 mM glucose, pH 7.4). Cells were preincubated in KRH buffer for 15 min at 37°C, and then [^{3}H]serotonin (14.3 Ci $mmol^{-1}$) was added and the incubation was carried out for 15 min at 37°C. The final concentration of serotonin was 50 nM. Both 100 μM pargyline and 100 μM L-ascorbic acid were added to the KRH buffer to prevent the degradation of serotonin. The uptake of [^{3}H]serotonin was terminated by washing the cells three times with ice-cold KRH buffer containing 50 μM imipramine. Cells were then solubilized with 1% SDS, and the radioactivity of the cell extracts was measured with a liquid scintillation counter (Beckman, LS6500).

2.6. Drug application

When the acute effects of drugs on serotonin transporter uptake activity were examined, the drugs were added in KRH-buffer during preincubation to obtain the desired concentration. For Northern blot analysis and for studying the long-term effects of drugs on serotonin transporter uptake activity, the drugs were directly applied to the culture medium. Fetal bovine serum was removed from the culture medium 24 h before the application of interferons. The mice were intraperitoneally injected with interferon-α or -γ (1×10^5 units kg^{-1} in saline) and were decapitated 3 h later. The brain and adrenal glands were dissected and the midbrain (approximately 50 mg) was further dissected with blades.

3. Results

3.1. Acute effects of interferon-α and -γ on the uptake activity of native and recombinant serotonin transporter

As shown in Fig. 1, serotonin was taken up into BeWo cells. The uptake of serotonin by BeWo cells was abolished by imipramine, a serotonin uptake inhibitor. The IC_{50} value of imipramine for serotonin uptake by BeWo cells was 0.32 μM. Eadie–Hofstee analysis revealed that the K_m value of imipramine-sensitive serotonin uptake in BeWo cells was 0.90 ± 0.26 μM (mean ± S.E.M., $n = 6$). The HeLa cells transfected with the serotonin transporter also accumulated serotonin, displaying pharmacological

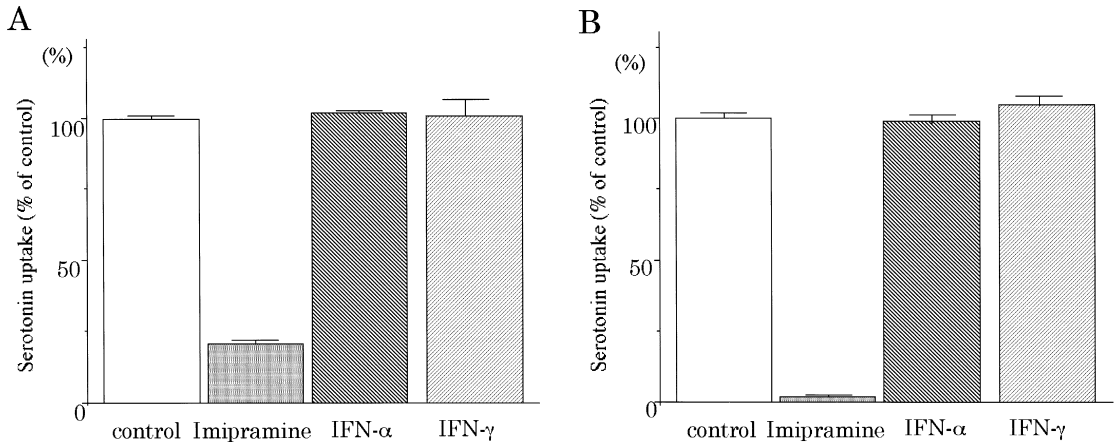

Fig. 1. Effects of short-term treatment with interferon-α and interferon-γ on the uptake of [^{3}H]serotonin by BeWo and serotonin transporter-transfected HeLa cells. Imipramine-sensitive serotonin uptake was observed both in BeWo (A) and serotonin transporter-transfected HeLa (B) cells. Neither interferon-α (IFN-α, 5000 units ml^{-1} 5×10^5 $cells^{-1}$) nor interferon-γ (IFN-γ, 5000 units ml^{-1} 5×10^5 $cells^{-1}$) affected serotonin uptake by BeWo cells or by serotonin transporter-transfected HeLa cells within 30 min after treatment. Data represent the means ± S.E.M. of six experiments.

properties similar to those described previously (Blakely et al., 1991; Hoffman et al., 1991).

Treatment with interferon-α (5000 units ml^{-1} 5×10^5 $cells^{-1}$) or interferon-γ (5000 units ml^{-1} 5×10^5 $cells^{-1}$) for 30 min did not affect the uptake of serotonin by BeWo cells. Similarly, serotonin uptake by HeLa cells transfected with the serotonin transporter was not altered by interferon-α or interferon-γ.

3.2. Effects of Dib-cAMP on the transcription of serotonin transporter in BeWo cells

Northern blotting analysis with rat serotonin transporter cDNA showed the steady-state expression of serotonin transporter mRNA in BeWo cells (Fig. 2A). The signals of serotonin transporter mRNA consisted of two bands: one intense band of 4.9 kb and a weak band of 3.0 kb (Fig. 2A). The treatment with Dib-cAMP (500 nmol ml^{-1} 5×10^5 $cells^{-1}$) markedly increased the level of serotonin transporter mRNA and the increase was comparable for both the 4.9 and 3.0 kb bands (Fig. 2A). The Dib-cAMP-induced increase in levels of serotonin transporter mRNA was time-dependent and an approximately 2-fold increase in levels of serotonin transporter mRNA was observed 24 h after treatment with Dib-cAMP. The increase in serotonin transporter mRNA induced by Dib-cAMP was blocked by simultaneous application of actinomycin D (30 ng ml^{-1} 5×10^5 $cells^{-1}$), an inhibitor of transcription (Fig. 2B). The treatment with actinomycin D alone induced a slight increase in serotonin transporter mRNA (103.0 ± 8.0 of the control, $n = 30$) but the increase was not significant. To determine whether serotonin transporter activity was increased due to the increase in serotonin transporter transcripts, serotonin uptake by BeWo cells was examined after treatment with Dib-cAMP. As shown in Fig. 2C, the uptake activity of the serotonin transporter was time dependently increased by Dib-cAMP (500 nmol ml^{-1} 5×10^5 $cells^{-1}$) and a 1.5-fold increase in serotonin uptake was observed 24 h after treatment. The time course of the Dib-cAMP-induced increase in serotonin uptake

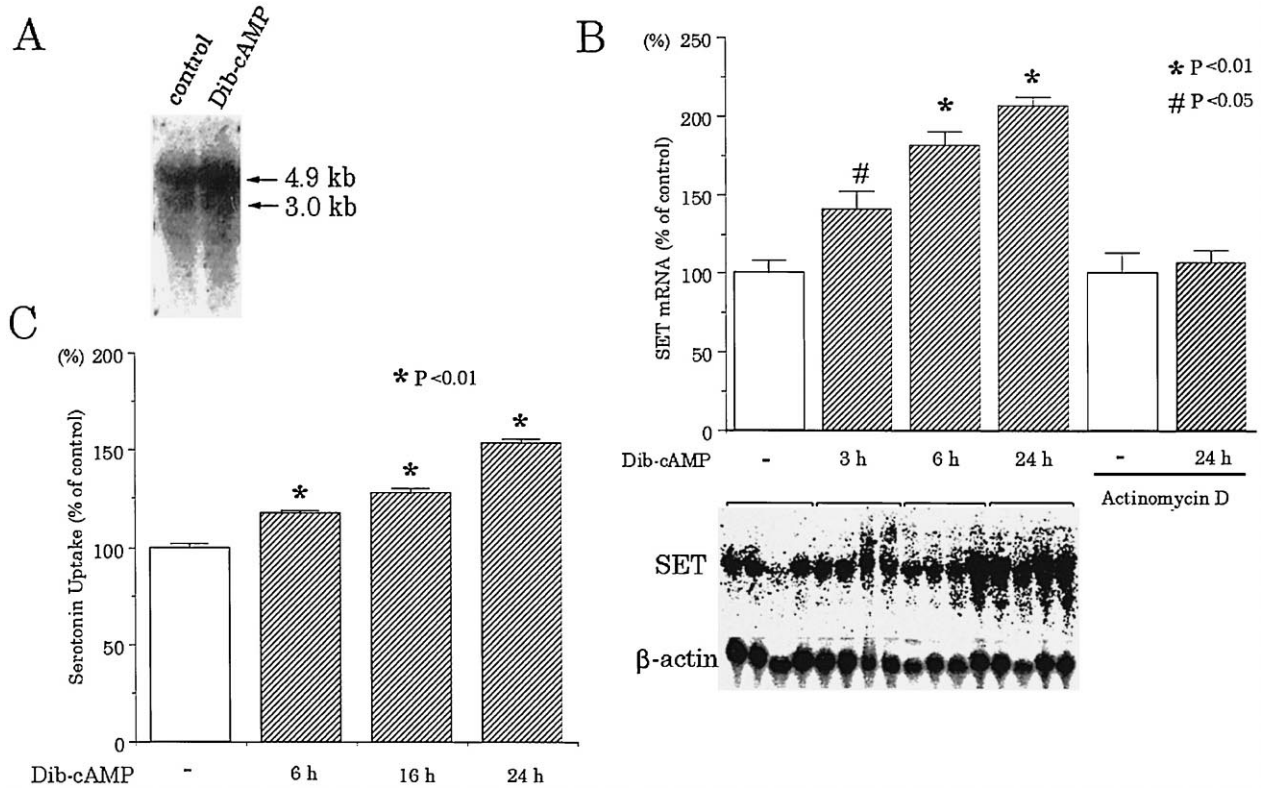

Fig. 2. Effects of Dib-cAMP on the transcription of serotonin transporter and on serotonin transporter activity in BeWo cells. (A) Northern blotting analysis with mRNA isolated from BeWo cells with serotonin transporter cDNA probes. The positions of the two serotonin transporter mRNAs of 4.9 and 3.0 kb are indicated at the right. Treatment with Dib-cAMP (500 nmol ml^{-1} 5×10^5 $cells^{-1}$) for 24 h markedly increased the signals for the serotonin transporter. (B) Effects of Dib-cAMP on the transcription of serotonin transporter (SET) in BeWo cells. The Dib-cAMP-induced increase in the level of serotonin transporter mRNA was time-dependent over 24 h and was blocked by simultaneous application of actinomycin D (30 ng ml^{-1} 5×10^5 $cells^{-1}$). The amount of mRNA was quantitated by measuring the intensity of phosphostimulated luminescence of each mRNA band, using Bio-Imaging Analyzer (Fuji, Japan). The data are expressed as ratios of the amount of serotonin transporter mRNA/β-actin mRNA, with the control value serotonin transporter at 100%. Data represent the means $\pm$ S.E.M. of four experiments. Statistical significance was calculated by using Student's t-test: $* P < 0.01$, $\#P < 0.05$. (C) Effects of Dib-cAMP on serotonin transporter activity in BeWo cells. The uptake activity of the serotonin transporter was time dependently increased by Dib-cAMP (500 nmol ml^{-1} 5×10^5 $cells^{-1}$). Data represent the means $\pm$ S.E.M. of six experiments. Statistical significance was calculated by using Student's t-test: $* P < 0.01$.

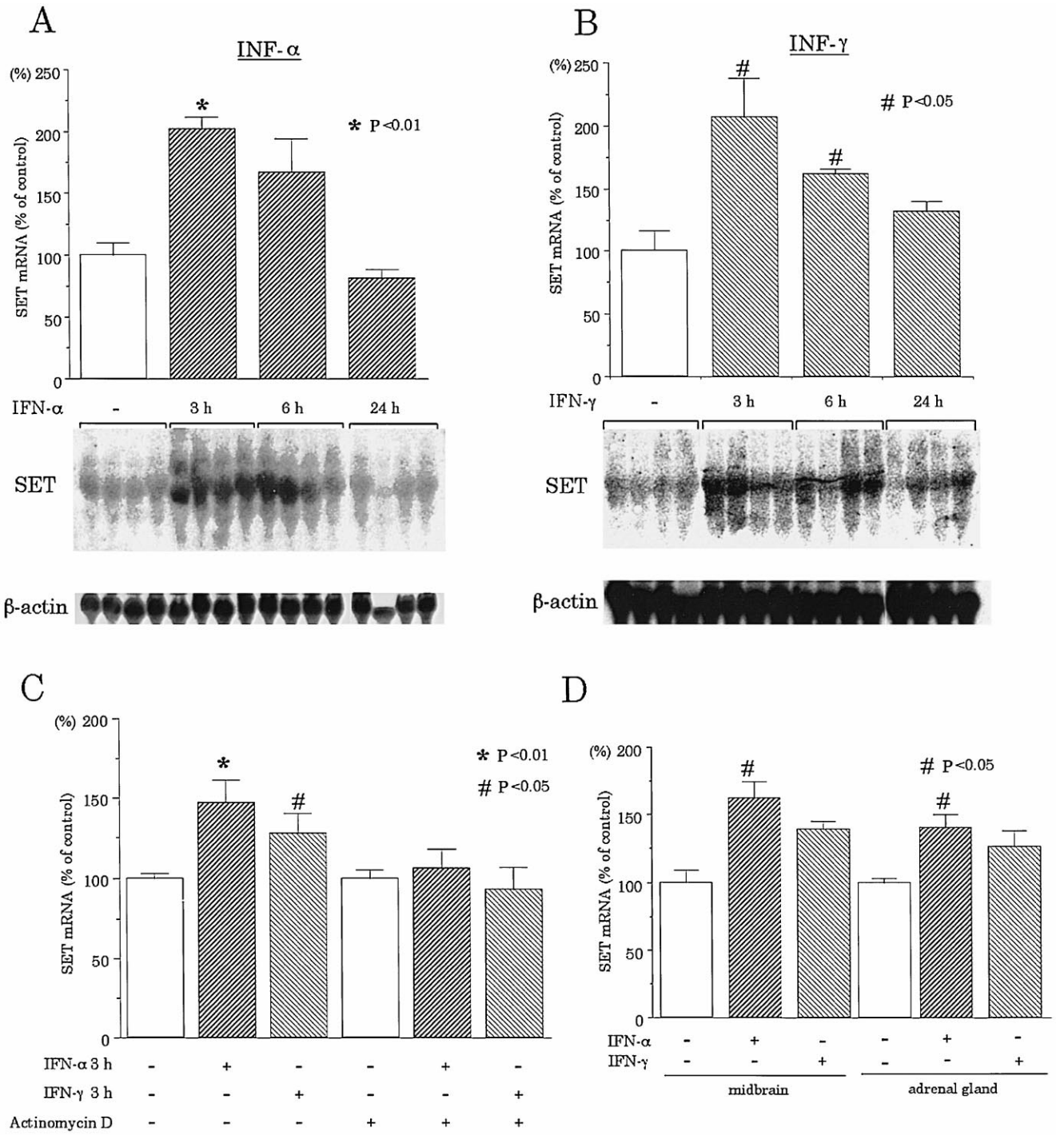

Fig. 3. Effects of interferon-α and -γ on the level of serotonin transporter (SET) mRNA in BeWo cells and in mice. (A) Time-course of the effects of interferon-α on the level of serotonin transporter mRNA in BeWo cells. Treatment with interferon-α (IFN-α, 5000 units ml^{-1} 5×10^5 $cells^{-1}$) significantly increased the level of serotonin transporter mRNA in BeWo cells. The increase reached maximum within 6 h. Data represent the means $\pm$ S.E.M. of four samples in a series of experiments. A statistically significant effect was obtained for interferon-α treatment. Statistical significance was calculated by using Student's *t*-test: $* P < 0.01$. (B) Time-course of the effects of interferon-γ on the level of serotonin transporter mRNA in BeWo cells. Interferon-γ (IFN-γ, 5000 units ml^{-1} 5×10^5 $cells^{-1}$) also significantly increased the level of serotonin transporter mRNA. The time dependence of this increase was similar to that of interferon-α. Data represent the means $\pm$ S.E.M. of four samples in a series of experiments. $\#P < 0.05$. (C) Effects of interferon-α and -γ on the level of serotonin transporter mRNA in BeWo cells. Both interferon-α and -γ significantly increased the level of serotonin transporter mRNA in BeWo cells. Data represent the means $\pm$ S.E.M. of more than 20 samples in five independent series of experiments. Actinomycin D (30 ng ml^{-1} 5×10^5 $cells^{-1}$) inhibited the increase in serotonin transporter RNA induced by both interferon-α and -γ. $* P < 0.01$, $\#P < 0.05$. (D) Effects of interferon-α and -γ on the level of serotonin transporter mRNA in mice. The levels of serotonin transporter mRNA in midbrain and adrenal gland were increased by the treatment with interferon-α and -γ (1×10^5 unit kg^{-1}). Data represent the means $\pm$ S.E.M. of more than four experiments. $\#P < 0.05$.

corresponded with the increased levels of serotonin transporter mRNA. Actinomycin D (30 ng ml^{-1} 5×10^5 $cells^{-1}$) also inhibited the Dib-cAMP-induced increase in serotonin uptake (data not shown).

3.3. Effects of interferon-α and -γ on levels of serotonin transporter mRNA in BeWo cells and in mice

Treatment with interferon-α (5000 units ml^{-1} 5×10^5 $cells^{-1}$) significantly increased the levels of serotonin transporter mRNA in BeWo cells (Fig. 3A). In a series of experiments, more than a 2-fold increase in the level of serotonin transporter mRNA was observed at 3 h and the mRNA level was enhanced by 1.7-fold at 6 h (Fig. 3A), whereafter levels returned to the basal level at 24 h. Interferon-γ (5000 units ml^{-1} 5×10^5 $cells^{-1}$) also significantly increased the level of serotonin transporter mRNA. The time-course of the increase in serotonin transporter mRNA induced by interferon-γ was similar to that induced by interferon-α (Fig. 3B). The maximum enhancement of serotonin transporter mRNA was observed 3–6 h after treatment and the mRNA level returned to the basal level after 24 h, but the magnitude of the increase varied in each experiment.

The increase in the levels of serotonin transporter mRNA 3 h after treatment with interferon-α or interferon-γ was further examined in other experiments and statistically analyzed. Both interferon-α and -γ significantly increased the levels of serotonin transporter mRNA by $47.3 \pm 14.4\%$ ($n = 20$) and $27.7 \pm 13.0\%$ ($n = 20$), respectively (Fig. 3C). Actinomycin D (30 ng ml^{-1} 5×10^5 $cells^{-1}$) inhibited the increase in serotonin transporter mRNA elicited by both interferon-α and -γ (Fig. 3C).

The regulation of serotonin transporter mRNA by interferons was also examined in vivo. Serotonin transporter mRNA was detected in the midbrain and in the adrenal gland of the rat. A 4.0-kb band was detected in the midbrain as well as in the adrenal gland. The levels of serotonin transporter mRNA both in the midbrain and adrenal gland were significantly increased by treatment with interferon-α for 3 h (Fig. 3D). Interferon-γ also increased the levels of serotonin transporter mRNA in the midbrain and adrenal gland, but the increase was not statistically significant.

3.4. Effects of long-term treatment with interferon-α and -γ on the uptake of serotonin by BeWo cells

While the treatment with interferon-α for 30 min did not affect the uptake of serotonin by BeWo cells (Fig. 1), treatment for 3 h or 6 h significantly increased it by 13.9 ± 3.3 and $9.6 \pm 1.4\%$, respectively (Fig. 4A). Actinomycin D (30 ng ml^{-1} 5×10^5 $cells^{-1}$) blocked the interferon-α-induced increase in serotonin uptake by BeWo cells (Fig. 4A). Similarly, treatment with interferon-γ for 3 and 6 h enhanced serotonin uptake by BeWo cells by 4.7 ± 1.1 and $10.5 \pm 3.3\%$, respectively (Fig. 4B). Actinomycin D (30 ng ml^{-1} 5×10^5 $cells^{-1}$) reversed the interferon-γ-induced increase in serotonin uptake by BeWo cells (Fig. 4B).

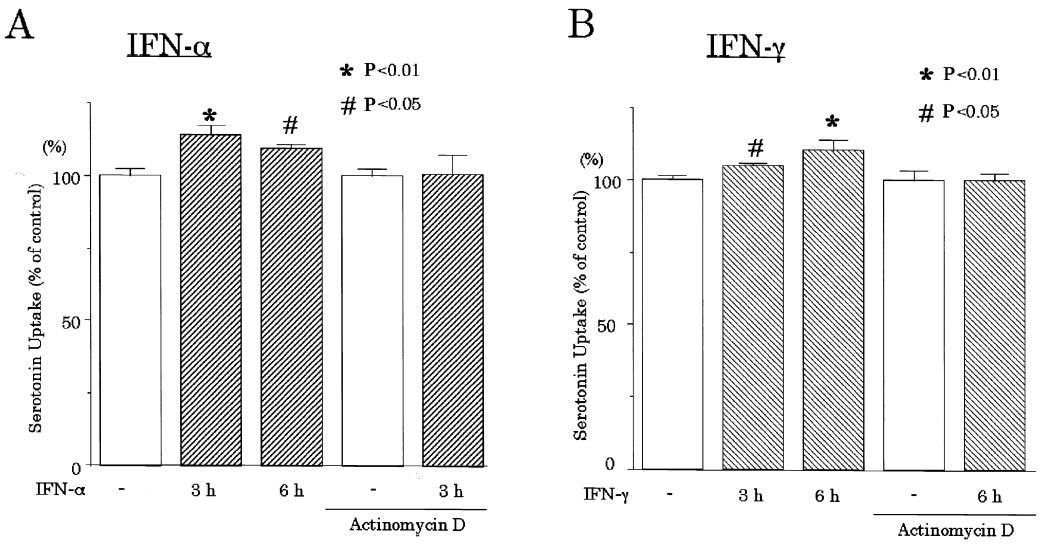

Fig. 4. Effects of longer treatment with interferon-α and interferon-γ on the serotonin uptake by BeWo cells. (A) Effects of interferon-α on serotonin uptake by BeWo cells. Treatment with interferon-α (IFN-α, 5000 units ml^{-1} 5×10^5 $cells^{-1}$) for 3 or 6 h slightly but significantly increased serotonin transporter activity. Actinomycin D (30 ng ml^{-1} 5×10^5 $cells^{-1}$) blocked the increase in serotonin transporter activity produced by interferon-α. Data represent the means ± S.E.M. of more than five experiments. Statistical significance was calculated by using Student's *t*-test: $* P < 0.01$, $\#P < 0.05$. (B) Effects of interferon-γ on serotonin uptake by BeWo cells. Treatment with interferon-γ (IFN-γ, 5000 units ml^{-1} 5×10^5 $cells^{-1}$) for 3 or 6 h slightly but significantly increased serotonin transporter activity. Actinomycin D (30 ng ml^{-1} 5×10^5 $cells^{-1}$) blocked the increase in serotonin transporter activity produced by interferon-γ. Data represent the means ± S.E.M. of more than five experiments. $* P < 0.01$, $\#P < 0.05$.

4. Discussion

Northern blot analysis revealed the presence of serotonin transporter mRNA in BeWo cells. The size of the mRNA (4.9 and 3.0 kb) in BeWo cells was the same as that detected in JAR cells (Cool et al., 1991; Ramamoorthy et al., 1995a), another choriocarcinoma cell line, but the 4.9-kb band was more intensely detected than the 3.0-kb band in the present experiments. In JAR cells, an additional 6.8-kb band was detected but we could not detect the third band in BeWo cells (Ramamoorthy et al., 1995a). This may be due to the different cell line or the different probe used for these experiments. The level of serotonin transporter mRNA in BeWo cells was increased by Dib-cAMP, an analogue of cAMP. The Na^+-dependent and imipramine-sensitive uptake of serotonin by BeWo cells was also increased by Dib-cAMP. These results were in agreement with previous findings for JAR cells (Ramamoorthy et al., 1993, 1995a) and showed that the serotonin transporter mRNA in BeWo cells is regulated by a mechanism similar to that observed in JAR cells.

Previous reports showed that the expression of various proteins in BeWo cells is modulated by interferons (Anderson and Berkowitz, 1985; Sekiya et al., 1986; Fulop et al., 1992; Hampson et al., 1993; Bennett et al., 1996), which strongly suggests that BeWo cells have the required pathways for stimulation by interferon-α and -γ, and that these cells express the receptors for interferons and their signaling pathway through Janus kinase–STAT. The level of serotonin transporter mRNA was increased by treatment with interferon-α and -γ and the effects were blocked by actinomycin D, which did not have significant effect on serotonin transporter mRNA. This finding strongly suggests that both interferon-α and -γ up-regulated the transcription of serotonin transporter. However, it is not clear whether the interferons cause this transcriptional regulation via the Janus kinase–STAT pathway. We examined the effects of genestein and lavendustin A, inhibitors of tyrosine kinase, including Janus kinase, on the interferon-induced transcriptional up-regulation of the serotonin transporter. Genestein and lavendustin A, however, both increased the level of serotonin transporter mRNA in BeWo cells (138.1 ± 16.4 and 244.0 ± 47.0% of control, respectively) significantly ($P < 0.05$ and $P < 0.01$ respectively) by unknown mechanisms. It is unclear, therefore, whether the transcriptional regulation of the serotonin transporter by interferon is blocked when these tyrosine kinase inhibitors are administered simultaneously.

The up-regulation of serotonin transporter mRNA by interferon treatment for 3 h caused a significant increase in the uptake of serotonin, and this increase in uptake was abolished by actinomycin D. It is suggested that the interferon-induced increase in serotonin transporter on the plasma membrane is due to the up-regulation of serotonin transporter transcription. The finding that treatment with interferons for a short time did not alter serotonin uptake suggests that the uptake function of the serotonin transporter is not enhanced by short-term interferon-mediated signaling, including the direct tyrosine phosphorylation of the serotonin transporter by Janus kinase. While the interferons induced a 1.5-fold increase in the level of serotonin transporter mRNA, the interferon-induced increase in the uptake activity of the serotonin transporter was small. It is possible that the half-life of the expressed serotonin transporter protein is long or that the amount of active serotonin transporter on the plasma membrane is increased only slightly although the translation of serotonin transporter was increased 1.5-fold.

The interferon-induced increase in serotonin transporter mRNA was transient and the maximum response was observed 6 h after treatment, while Dib-cAMP continuously increased levels of serotonin transporter mRNA for up to 24 h. This suggests that the regulatory mechanism of serotonin transporter transcription by interferons differs from that exerted by cAMP and that the transcriptional regulation mediated by interferons is not sustained. A similar transcriptional up-regulation by interferons has also been reported: the transcription of platelet-activating factor (PAF) receptor and the platelet factor IV family of cytokines is up-regulated by interferon-γ and the enhancement of transcription reaches a maximum within 8 h (Farber, 1990; Ouellet et al., 1994).

Both interferons, but especially interferon-α, increased the level of serotonin transporter mRNA in the midbrain and adrenal gland from interferon-treated mice. The midbrain contains the dorsal raphe nuclei, which is the only area in the brain that expresses serotonin transporter mRNA (Blakely et al., 1991). This suggests that the interferons act to modulate the transcription of serotonin transporter in vivo. Although an interferon responsive sequence has not been reported in the promoter region of the human serotonin transporter gene, in our preliminary study, the consensus binding sites of STAT family members were detected in the 5′-flanking region of the mouse serotonin transporter gene (data not shown). This suggests that, at least in mice, interferons increase the transcription of serotonin transporter mRNA from its gene via the Janus kinase–STAT pathway. Long-term treatment with interferon may increase the transcription of serotonin transporter, enhance the uptake of serotonin in serotonergic synapses, and terminate transmission rapidly.

Considering that serotonin-specific reuptake inhibitor is effective against interferon-induced psychiatric side effects (Levenson and Fallon, 1993), the interferon-induced depression is probably due to an imbalance of serotonin levels in the synaptic terminal. It is noteworthy that the effect of serotonin-specific reuptake inhibitor on interferon-induced complications is delayed for 6 weeks after the administration of antidepressants and the clinical therapeutic effects of antidepressants are delayed by 2 to 3 weeks (Baldessarini, 1980), though their actions on monoamine uptake are immediate. Such delays suggest

that compensatory changes occur downstream from the serotonin uptake-blocking effect, and that such changes develop more slowly for interferon-induced depression. It is possible that transcriptional regulation of the serotonin transporter is a neurochemical mechanism underlying affective disorders.

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