ELSEVIER

Brain Research 727 (1996) 118–124

BRAIN RESEARCH

Research report

Hydroxyindole-*O*-methyltransferase in Y-79 cells: regulation by serum

Marianne Bernard [a], Pierre Voisin [b], David C. Klein [a,*]

[a] *Section on Neuroendocrinology, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA*

[b] *Laboratoire de Biologie Cellulaire, URA CNRS 1869, 40 avenue du Recteur Pineau, F-86022 Poitiers Cedex, France*

Accepted 12 March 1996

Abstract

Hydroxyindole-*O*-methyltransferase (HIOMT) catalyzes the last step in the synthesis of melatonin. In the present study, the regulation of HIOMT expression was examined in the human Y-79 retinoblastoma cell line. Cells were grown in suspension culture using medium supplemented with 10% fetal calf serum (FCS). HIOMT activity and mRNA were strongly reduced when FCS was substituted with 0.1% bovine serum albumin (BSA), and were restored by addition of FCS. The effect of FCS on HIOMT expression was relatively selective, because the abundance of mRNA encoding actin, G3PDH or interphotoreceptor retinoid-binding protein did not change following serum deprivation. However, S-antigen (arrestin) mRNA was regulated by serum coordinately with HIOMT mRNA, suggesting that S-antigen expression is also controlled by a serum factor. The effect of serum on HIOMT expression was not duplicated by treatment with a series of known differentiating factors, nor was it reduced by dialysis or stripping procedures which remove steroids, growth factors and thyroid hormones.

Keywords: Hydroxyindole-*O*-methyltransferase; Melatonin; Y-79 retinoblastoma; Serum; S-antigen

1. Introduction

Melatonin plays a key role in the photoneuroendocrine system, functioning as a signal of darkness. It mediates the global changes in physiology which occur in many species on a seasonal basis, including changes in behavior, reproduction, and weight gain. Melatonin is synthesized from tryptophan by a multi-enzyme pathway. The last enzyme, hydroxyindole-*O*-methyltransferase (HIOMT; EC 2.1.1.4; [4,28]) appears to limit melatonin production at night, when the level of the HIOMT substrate *N*-acetylserotonin is maximal [17]. HIOMT is found consistently at high levels only in the pineal gland, the source of circulating melatonin [5,6].

HIOMT has also been found in some vertebrate retinae [7,12,22,29] and in the Y-79 human retinoblastoma cell line [15]. In a recent report, we have shown that the characteristics of HIOMT expression in Y-79 cells are essentially the same as in the pineal gland, although the level of expression is approximately 20-fold lower [9]. The Y-79 cell line is also known to express members of the cassette of proteins normally found only in pinealocytes and/or retinal photoreceptors [10,15,18,21]. Accordingly, these cells are of special value because they provide an experimental model to study how the expression of these pineal/retina specific proteins is regulated.

Relatively little is known about the signals which control tissue-specific and developmental expression of HIOMT. Physiological regulation of HIOMT expression in the mammalian pineal gland appears to involve neural and non-neural factors [26,27,30]. In the adult rat, nocturnal stimulation of the pineal gland by the suprachiasmatic nuclei (via the superior cervical ganglia) maintains constant levels of HIOMT activity [16]. This appears to involve a β-adrenergic mechanism at the level of the pineal gland [27]. However, neural stimulation does not seem to be required for triggering the development of HIOMT activity in newborn rats [26]. Studies on the regulation of avian pineal HIOMT indicate that developmental expression of the enzyme might be under hormonal control [19] and that light and a circadian oscillator control marked day/night changes in HIOMT mRNA [8].

Presently, little is known about the factors that control HIOMT gene expression in human Y-79 cells. Previous studies have shown that these cells can differentiate into

* Corresponding author. Present address: Building 49, Room 5A38, MSC 4480, National Institutes of Health, Bethesda, MD 20892, USA. Fax: (1) (301) 480-3526.

0006-8993/96/$15.00 Published by Elsevier Science B.V.
PII S0006-8993(96)00359-9

either a conventional neuron- or a photoreceptor-like phenotype, depending on culture conditions [1]. In the current investigation, we examined another aspect of photoreceptor-like differentiation – melatonin synthesis – by analyzing the regulation of the melatonin-synthesizing enzyme HIOMT. Our data show that the basal expression of HIOMT in Y-79 cells is controlled by a serum factor in a relatively selective manner.

2. Materials and methods

2.1. Materials

The following were purchased. Heat-inactivated fetal calf serum, RPMI 1640 (Biofluids, Rockville, MD); AG1-X8 (Cl^-) resin (Bio-Rad, Hercules, CA); [^{3}H-methyl]*S*-adenosyl-L-methionine, [α-^{32}P]deoxycytidine 5′-triphosphate (dCTP), Megaprime DNA labelling kit (Amersham, Arlington Heights, IL); bovine serum albumin Cohn fraction V (BSA) (Intergen, Purchase, NY); acid-washed activated charcoal, N^6,2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate (DB-cAMP), dexamethasone, hydrocortisone, *N*-acetylserotonin, norepinephrine bitartrate-salt, *S*-adenosyl-L-methionine (Sigma, St. Louis, MO); QuikHyb (Stratagene, La Jolla, CA); fetal bovine serum dialyzed, calf serum dialyzed, human recombinant basic fibroblast growth factor (bFGF), medium supplements (N-2, G-5 and GMS-G), Trizol™ (Gibco-BRL, Gaithersburg, MD); human recombinant somatotropin (Boehringer, Indianapolis, IN); L-3,3′,5-triiodothyronine (T3) (Calbiochem, San Diego, CA); SeaKem GTG (FMC BioProducts, Rockland, ME); Nytran (Schleicher and Schuell, Keene, NH).

2.2. Methods

2.2.1. Cell culture

Y-79 retinoblastoma cells (American Type Tissue Culture; Rockville, MD) were maintained in suspension culture at 37°C (95% air, 5% CO_2) in RPMI 1640 medium supplemented with 2 mM glutamine, 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. The density of cells was kept between 5×10^5 and 10^6 cells per ml. Under these conditions cells tend to form large aggregates.

For experimental treatments, cells were collected by centrifugation and resuspended in medium containing serum (10%), or BSA (1 mg/ml) alone or combined with medium supplements. Somatotropin, dibutyryl-cAMP, bFGF, hydrocortisone (water-soluble) and norepinephrine were dissolved directly in culture medium; T3 and dexamethasone were first dissolved in dimethyl sulfoxide (DMSO) and then diluted in culture medium. The composition of the commercial supplements was: N-2 = 5 μg/ml insulin, 100 μg/ml transferrin, 5.2 ng/ml sodium selenite, 6.3 ng/ml progesterone, 16 μg/ml putrescine; G-5 = 5 μg/ml insulin, 50 μg/ml transferrin, 5.2 ng/ml sodium selenite, 10 ng/ml biotin, 3.6 ng/ml hydrocortisone, 5 ng/ml FGF, 10 ng/ml EGF; GMS-G = 10 μg/ml insulin, 5 μg/ml transferrin, 6.7 ng/ml sodium selenite.

Sequential adsorption of fetal calf serum on anion-ex-

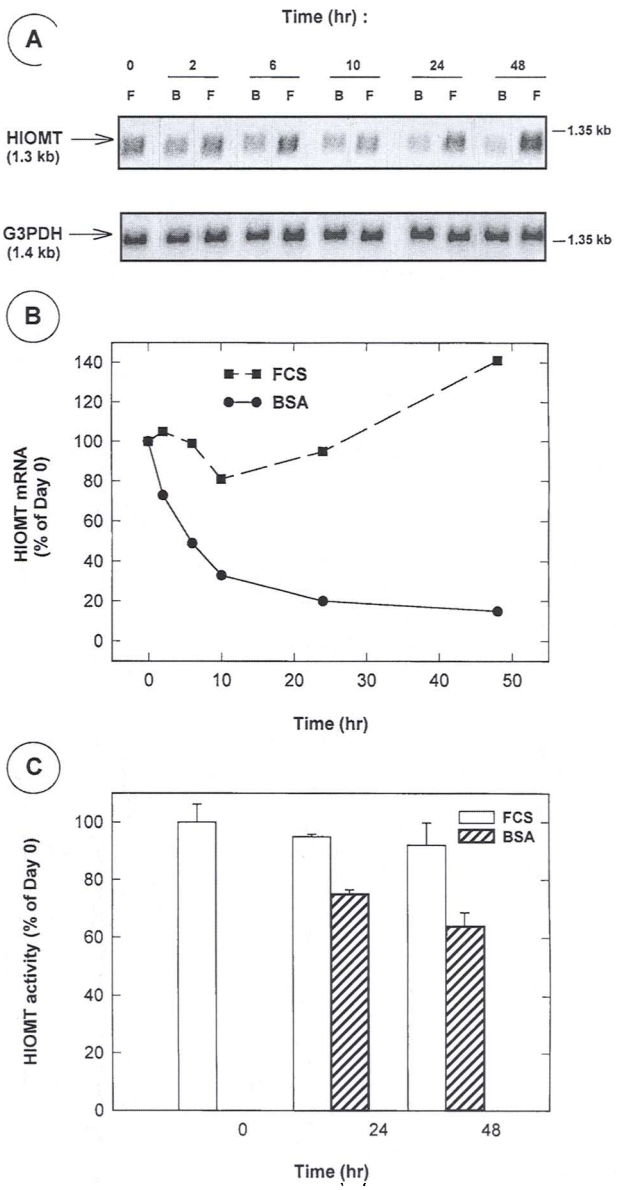

Fig. 1. HIOMT mRNA and enzyme activity levels in Y-79 cells cultured in the presence or absence of FCS. Y-79 cells that had been maintained in RPMI 1640 supplemented with 10% FCS were cultured for 2 days in media containing either 10% FCS (F) or 0.1% BSA (B). At the indicated times, cells were collected by centrifugation and processed for RNA analysis or enzyme assay. A: Northern blot analysis of HIOMT and G3PDH mRNA (20 μg of total RNA/lane). B: densitometric analysis of the Northern blot. The HIOMT values in individual samples have been normalized to the G3PDH signal and are expressed as percentage of the value before transferring the cells into serum-free medium (day 0). Results are the mean of two separate experiments, both performed in duplicate. Individual values were within 20% of the mean. C: HIOMT activity. Values represent the mean ± S.E.M. (n = 3) and are expressed as percentage of the value measured at day 0. For further details see Section 2.

change resin AG1-X8 (Cl^-) and activated charcoal was performed as described by Smith et al. [25]. Alternatively, aliquots of serum were adsorbed only on resin or only on charcoal.

2.2.2. Enzyme assays

HIOMT activity was determined radiochemically by incubating (30 min; 37°C) a cell extract prepared from approximately 10^6 cells with 50 μM *N*-acetylserotonin (NAS) and 2.5 μM [^{3}H-methyl]*S*-adenosyl-L-methionine (500 Ci/mol; SAM; [4,28]). The assay is linear with 0.5×10^6 cells for 40 min, and is linear with time for 30 min with up to 10^6 cells.

Serotonin *N*-acetyltransferase (NAT; EC 2.3.1.87) activity was determined radiochemically [20]. The concentrations of substrate and cosubstrate in the assay were 10 mM tryptamine and 425 μM [acetyl-^{3}H]acetyl-CoA (10 Ci/mol).

2.2.3. Protein assay

Protein was measured using a dye-binding method [11] with BSA as standard.

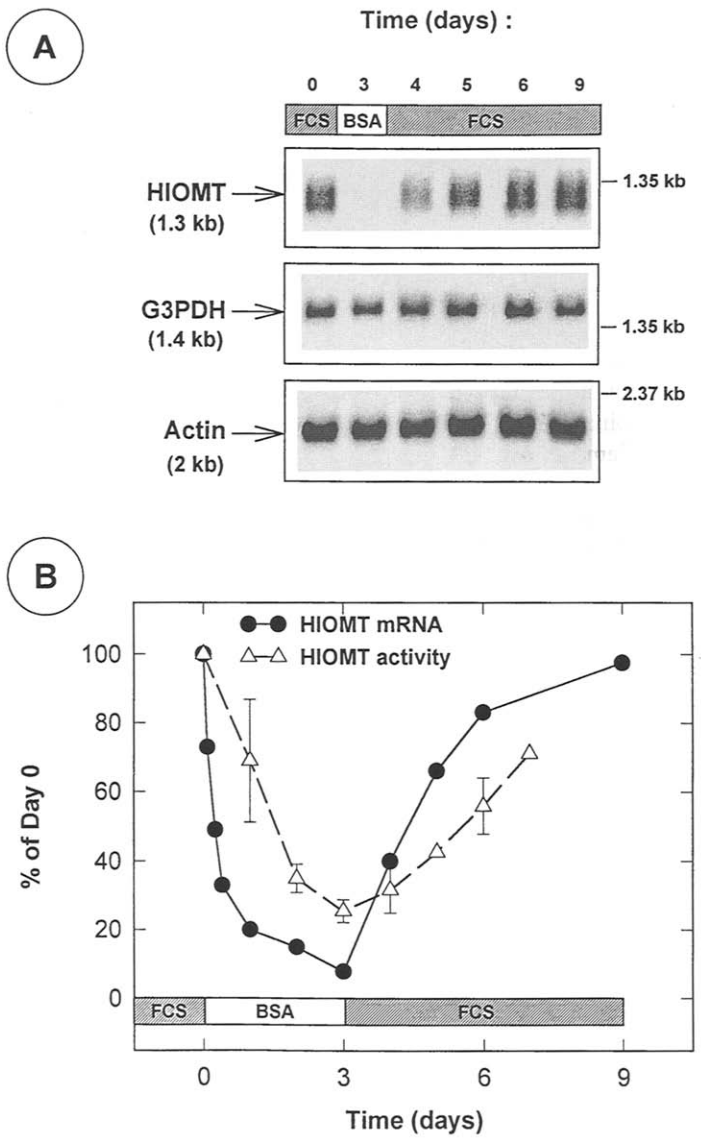

Fig. 2. The effect of serum deprivation on HIOMT enzyme activity and mRNA is reversible. Cells that had been maintained in RPMI/10% FCS medium were cultured for 3 days in serum-free RPMI containing 0.1% BSA, and were then transferred for 6 days in medium supplemented with FCS (10%). Cells were harvested at the indicated times and were processed for RNA analysis or enzyme assay. A: Northern blot analysis of HIOMT, G3PDH and actin mRNA was performed (20 μg of total RNA/lane). B: densitometric analysis of the Northern blot (●). The values of HIOMT mRNA in individual samples have been normalized to the G3PDH signal and are the mean of two separate experiments, both performed in duplicate. Individual values were within 15% of the mean. The values of HIOMT enzyme activity represent the mean ± S.E.M. of three separate experiments (△). For both HIOMT mRNA and HIOMT enzyme activity, results have been expressed as percentage of the values before transferring the cells into serum-free medium (day 0). For further details see Section 2.

2.2.4. Northern blot analysis of RNA

Extraction of mRNA. Total RNA was isolated using Trizol™ reagent as described by the manufacturer. Total RNA was fractionated on 1.5% agarose/0.66 M formaldehyde gel and subsequently transferred to nylon membrane by capillary blotting with 20 × SSC (3 M NaCl and 0.3 M sodium citrate). RNA was cross-linked to the membrane in a UV oven (Stratagene, La Jolla, CA; 120 mJ, 35 s).

Probes. The probe used for detection of HIOMT mRNA on Northern blots was 5′-HIOMT, a 473 bp-5′ fragment of the human HIOMT cDNA (bases 28–500; [13]); this does not include the truncated LINE-1 sequence found in one isoform of HIOMT mRNA. A 2 kb probe for the human β-actin and a 1.1 kb probe for the human glyceraldehyde-3-phosphate-dehydrogenase (G3PDH; Clontech, Palo Alto, CA) were used to monitor the quality of RNA preparations and to normalize the HIOMT signal.

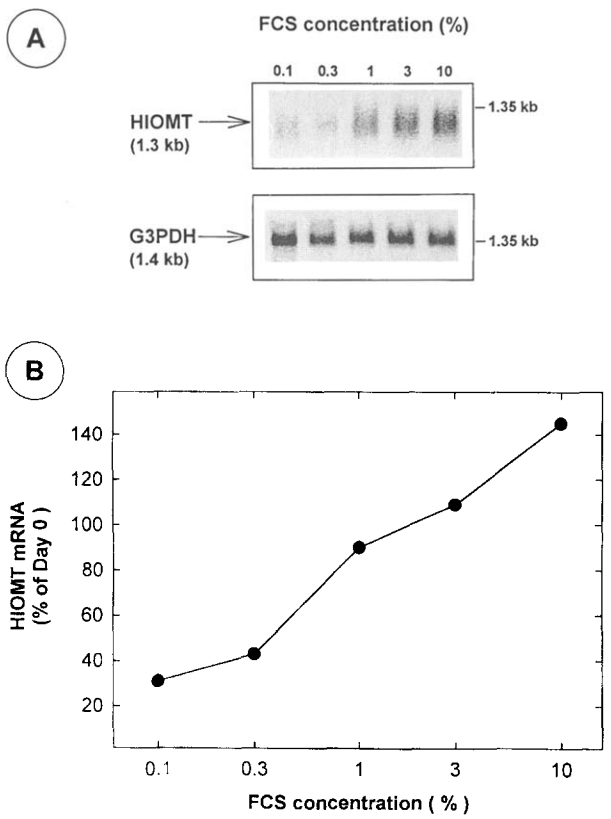

Fig. 3. Concentration dependency of the effect of FCS. Y-79 cells that had been maintained in RPMI medium supplemented with 10% FCS were cultured for 3 days in serum-free RPMI containing 0.1% BSA and were then transferred for 3 days in medium supplemented with the indicated concentrations of FCS. A: cells were harvested and processed for Northern blot analysis of HIOMT and G3PDH mRNA (20 μg of total RNA/lane). B: densitometric analysis of the Northern blot. The HIOMT mRNA values in individual samples have been normalized to the G3PDH signal and are expressed as percentage of the values before transferring the cells into serum-free medium (day 0). Results are the mean of duplicate determinations; individual values were within 20% of the mean. Similar results were obtained with 10% and 1% FCS in four independent experiments. For further details see Section 2.

A 702 bp probe for human S-antigen (arrestin) was prepared by PCR amplification of human retinal cDNA using primers specific for the human S-antigen coding sequence (GenBank/EMBL Data Bank, accession number X12453): 5′ primer = GAGGACGTTGACGTGATCG (bp 441–459); 3′ primer = GTCCTCGTGCTTGATTTTCC (bp 1142–1123). A 472 bp probe for human interphotoreceptor retinoid-binding protein (IRBP) was prepared by PCR amplification of human retinal cDNA using primers specific for the human IRBP coding sequence (GenBank/EMBL Data Bank, accession number M22453): 5′ primer = CACCAACCTCTACCTCACTATCCCC (bp 3670–3694); 3′ primer = TGGTAGAACTTGGGGTGGTATTTTT (bp 4141–4117). Probes were labeled with [α-^{32}P]dCTP (3000 Ci/mmol) by random priming (Megaprime kit; Amersham, Arlington Heights, IL).

Analysis. Blots were incubated in prehybridizing solution (Quikhyb; Stratagene, La Jolla, CA) for 15 min at 68°C, ^{32}P-labeled probe was added (10^6 cpm/ml of either HIOMT, β-actin, G3PDH, S-antigen or IRBP probe) and the 68°C incubation was continued for 1.5 h. After hybridization, the blots were washed twice (15 min each at room temperature) in 2 × SSC/0.1% SDS and once (30 min at 42°C for HIOMT, S-antigen and IRBP probes, or 60°C for β-actin and G3PDH probes) in 0.1 × SSC/0.1% SDS. Blots were exposed overnight to a PhosphorImager screen and band intensities were measured by integration of area under the peaks of interest (Image Quant; Molecular Dynamics, Sunnyvale, CA). Typically, blots were first hybridized with 5′-HIOMT probe and subsequently stripped (twice 15 min in water ≤ 100°C) before hybridization with G3PDH or actin probe. In some cases, blots were stripped again and probed with another ^{32}P-labeled cDNA.

3. Results

The levels of HIOMT mRNA and enzyme activity in Y-79 cells remain relatively stable in the presence of 10% FCS (Fig. 1). Replacement of FCS with BSA resulted in a marked reduction in both HIOMT mRNA and enzyme activity (Fig. 1A, C). The abundance of HIOMT mRNA decreases 80% in two days, with a half-life of approximately 5 h (Fig. 1B, Fig. 2B). During the same period HIOMT activity decreased 40 to 60%, with a half-life of 24 h (Fig. 1C and Fig. 2B). This decrease in HIOMT expression was not due to an inhibitory agent contaminating the BSA preparation, because addition of BSA to the serum-supplemented medium did not decrease HIOMT mRNA (data not shown).

This decrease in HIOMT mRNA and enzyme activity was reversed by addition of FCS (Fig. 2), indicating that serum deprivation did not result in death of the responsive cells or cause irreversible loss of responsiveness. The EC_{50} of this effect was ~ 1% (Fig. 3).

To further examine the specificity and selectivity of the effect of serum deprivation, several criteria were examined. First, trypan blue exclusion indicated that cell survival did not change due to serum deprivation, although analysis of cell growth indicated that the doubling time increased from ~36 h to ~72 h following serum removal (data not shown). Second, measurement of G3PDH mRNA and actin mRNA (Fig. 1A and Fig. 2A) indicated that these parameters were not affected by the absence of serum.

In addition to these non-specific cell markers, more specific indices of Y-79 function were also examined. Northern blot analysis of other mRNA species revealed that serum deprivation did not reduce the abundance of IRBP mRNA, a marker of early retinal differentiation (Fig. 4), but it did reduce the abundance of S-antigen mRNA (Fig. 4), a protein involved in phototransduction. Similarly to the effect on HIOMT mRNA, the decrease in S-antigen mRNA induced by serum deprivation was reversed by addition of FCS (Fig. 4). Finally, measurement of serotonin *N*-acetyltransferase (NAT) activity, the penultimate enzyme in melatonin synthesis, indicated that it did not decrease following 3 days in serum-free medium; and, it was still possible to elevate NAT activity by DB-cAMP treatment (data not shown). These observations indicate that several biochemical markers of Y-79 cells are not changed by serum deprivation.

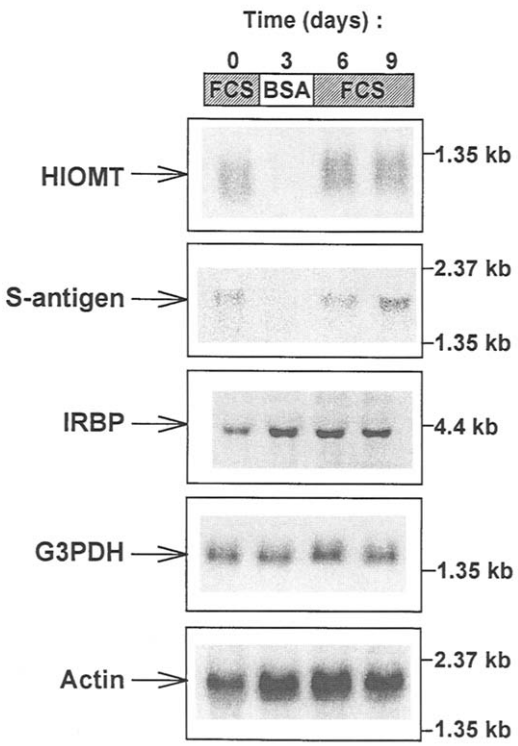

Fig. 4. The effect of FCS on HIOMT mRNA is relatively selective. Y-79 cells that had been maintained in RPMI medium supplemented with 10% FCS were cultured for 3 days in RPMI containing 0.1% BSA and were then transferred in RPMI/10% FCS. Cells were harvested and the levels of mRNA encoding HIOMT, actin, G3PDH, IRBP and S-antigen were determined by Northern blot analysis (20 μg of total mRNA/lane). Similar results were obtained in three independent experiments, each performed in duplicate. For further details see Section 2.

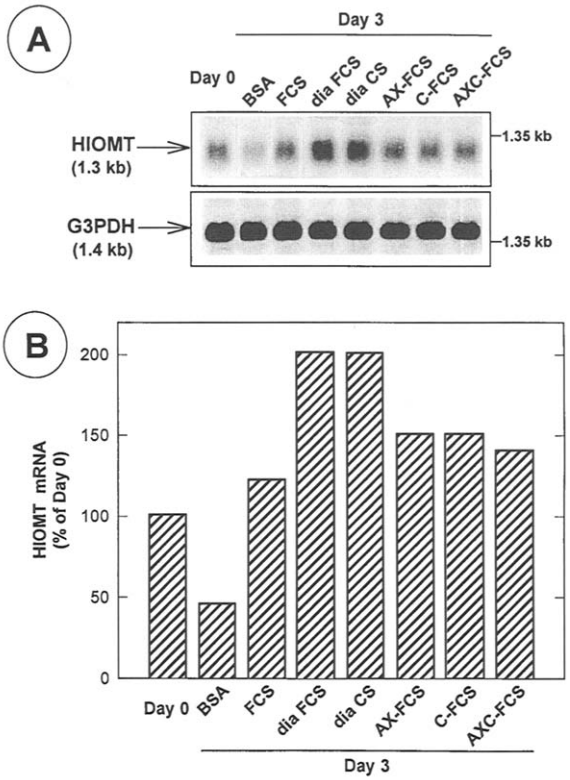

Fig. 5. Comparison of the effects of whole versus dialyzed or stripped bovine sera, on HIOMT mRNA levels. A: Y-79 cells were cultured for 3 days in RPMI containing 0.1% BSA or 10% of the indicated type of bovine serum. FCS, whole fetal calf serum; dia FCS, dialyzed FCS; dia CS, dialyzed newborn-calf serum; AX-FCS, FCS adsorbed on AG1-X8 resin; C-FCS, FCS adsorbed on activated charcoal; AXC-FCS, FCS adsorbed on both AG1-X8 and activated charcoal. Cells were harvested and HIOMT mRNA levels were determined by Northern blot analysis (20 μg of total RNA/lane). B: densitometric analysis of the Northern blot. The values of HIOMT mRNA have been normalized to the G3PDH signal and are expressed as percentage of the values before transferring the cells into serum-free medium. Results are the mean of duplicate determinations; individual values were within 20% of the mean. Similar results were obtained in a second experiment. For further details see Section 2.

Two different approaches were used to determine if small molecular mass compounds were responsible for the effects of serum. First, Y-79 cells were cultured for 3 days in serum-free media supplemented with commercial medium supplements (N-2, G-5, GMS-G) and/or selected agents known to play a role in cell differentiation in other systems (DB-cAMP, norepinephrine, basic FGF, dexamethasone, T3, somatotropin and hydrocortisone). None of these prevented the decrease in HIOMT mRNA. Secondly, dialysis and several stripping methods which eliminate steroids, thyroid hormones and growth factors [24,25] were used. None of these treatments reduced the effectiveness of serum (Fig. 5).

4. Discussion

The findings presented here indicate that basal expression of HIOMT in Y-79 cells is strongly dependent upon the presence of FCS in the culture medium. This suggests that expression of the human HIOMT gene might be controlled by a serum factor.

Several lines of evidence indicate that the effects of serum deprivation on HIOMT activity and mRNA are relatively selective. First, the serum-deprived cells did not exhibit a marked decrease in cell viability. Second, the decrease in HIOMT expression was reversible by serum replacement. Third, the effect of serum was relatively selective because the levels of actin, G3DPH and IRBP mRNAs, as well as NAT activity remained unchanged in serum-deprived cells, in contrast to HIOMT. These findings indicate that the effect of serum on HIOMT does not reflect global changes in cell function. The observation that effects of serum on HIOMT were paralleled by similar changes in S-antigen raises the possibility that the same compounds might be involved in the regulation of both genes. It is possible that this reflects an action of serum which is linked to tissue-specific developmental gene expression. However, it is not clear whether this involves a single molecule in serum or if each gene is influenced by a different agent.

This influence of serum on HIOMT appears to reflect the action of a compound which is not freely diffusible, because dialyzed bovine sera were as effective as whole FCS in preventing the decrease in HIOMT mRNA. This suggests that the serum factor involved might be either a protein or a small molecule tightly bound to a protein. Our first attempt to identify this factor has been unsuccessful. Factors that have previously been shown to play a role in HIOMT expression in the chicken embryo (thyroxine, somatotropin and hydrocortisone; [19]) or to trigger the differentiation of embryonic rat retinal cells into photoreceptors (basic FGF; [14]) do not appear to be involved in the basal expression of human HIOMT in Y-79 cells. The lack of involvement of these compounds is also consistent with the results of stripping experiments which removed steroids, thyroid hormones and growth factors.

Although the analysis of the promoter region of the human HIOMT gene has indicated the presence of a potential cAMP regulatory element [23], stimulation of Y-79 cells with DB-cAMP did not increase the abundance of HIOMT mRNA. This is in agreement with a previous study in which HIOMT enzyme activity in Y-79 cells was not stimulated by forskolin, an agent which elevates intracellular cAMP levels [15]. This indicates that the serum component which controls HIOMT expression does not act through the cAMP second messenger pathway.

The findings of factors controlling HIOMT gene expression might be of some clinical relevance, because it has been noted that there are marked individual-to-individual variations in melatonin production in humans [2,3]. In separate studies it has been found that HIOMT activity and immunoreactivity in post-mortem pineal glands also varies markedly [9,13]. This points to the possibility that variations in HIOMT expression might be responsible for the large individual-to-individual variations in melatonin production, and that the serum factor which regulates HIOMT gene products in Y-79 cells might be involved in the physiological regulation of melatonin production. This serum factor could be hormonal or nutritional, which raises the possibility that previously unsuspected agents might control HIOMT activity and, through this action, modulate melatonin production. Identification of such an agent will be of interest because it might have some clinical utility.

References

[1] Albini, A., Noonan, D.M., Melchiori, A., Fassina, G.A., Percario, M., Gentleman, S., Toffenetti, J. and Chader, G.J., Laminin-induced retinoblastoma cell differentiation: possible involvement of a 100-kDa cell-surface laminin-binding protein. *Proc. Natl. Acad. Sci. USA*, 89 (1992) 2257–2261.

[2] Arendt, J., Melatonin and the human circadian system. In A. Miles et al. (Eds.), *Melatonin: Clinical Perspectives*, Oxford Medical Publications, Oxford, UK, 1988, pp. 43–61.

[3] Arendt, J., Wetterberg, L., Heyden, T., Sizonenko, P.C. and Paunier, L., Radioimmunoassay of melatonin: Human serum and cerebrospinal fluid, *Hormone Res.*, 8 (1977) 65–75.

[4] Axelrod, J. and Weissbach, H., Enzymatic O-methylation of N-acetylserotonin to melatonin, *Science*, 131 (1960) 1312.

[5] Axelrod, J. and Weissbach, H., Purification and properties of hydroxyindole-*O*-methyltransferase, *J. Biol. Chem.*, 236 (1961) 211–213.

[6] Axelrod, J., Maclean, P.D., Albers, R.W. and Weissbach, H., Regional distribution of methyltransferase enzymes in the nervous system and glandular tissues. In S.S. Kety and J. Elkes (Eds.), *Regional Neurochemistry*, Pergamon Press, New York, 1961, pp 307.

[7] Baker, P.C., Quay, W.B. and Axelrod, J., Development of hydroxyindole-*O*-methyltransferase activity in eye and brain of the amphibian, *Xenopus laevis*, *Life Sci.* 4 (1965) 1981–1984.

[8] Bernard, M., Guerlotté, J., Cogné, M., Grève, P., Collin, J.P. and Voisin, P., Transcriptional regulation of hydroxyindole-*O*-methyltransferase in the chicken pineal gland: day/night changes and long-term effect of light and darkness, *Biochem. J.*, 290 (1993) 661–664.

[9] Bernard, M., Donohue, S. and Klein, D.C., Human hydroxyindole-*O*-methyltransferase in pineal gland, retina and Y-79 retinoblastoma cells, *Brain Res.*, 696 (1996) 37–48.

[10] Bogenmann, E., Lochrie, M.A. and Simon, M.I., Cone cell-specific genes expressed in retinoblastoma, *Science*, 240 (1988) 76–78.

[11] Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding, *Anal. Biochem.*, 72 (1976) 248–254.

[12] Cardinali, D.P. and Rosner, J.M., Retinal localization of the hydroxyindole-*O*-methyltransferase (HIOMT) in the rat, *Endocrinology*, 89 (1971) 301–305.

[13] Donohue, S.J., Roseboom, P.H. Illnerova, H., Weller, J. and Klein, D.C., Human hydroxyindole-*O*-methyltransferase: Presence of LINE-1 fragment in a cDNA clone and pineal mRNA, *DNA Cell Biol.*, 12 (1993) 715–727.

[14] Hicks, D. and Courtois, Y., Fibroblast growth factor stimulates photoreceptor differentiation in vitro, *J. Neurosci.*, 12 (1992) 2022–2033.

[15] Janavs, J.L., Pierce, M.E. and Takahashi J., *N*-acetyltransferase and protein synthesis modulate melatonin production by Y-79 human retinoblastoma cells, *Brain Res.*, 540 (1991) 138–144.

[16] Klein, D.C. and Moore, R.Y., Pineal N-acetyltransferase and hydroxyindole-*O*-methyltransferase: control by the retinohypothalamic tract and the suprachiasmatic nucleus, *Brain Res.*, 174 (1979) 245–262.

[17] Klein, D.C. and Weller, J.L., Indole metabolism in the pineal gland: A circadian rhythm in N-acetyltransferase, *Science*, 169 (1970) 1093–1095.

[18] Kyritsis, A.P., Wiggert, B., Lee, L. and Chader, G.J., Butyrate enhances the synthesis of interphotoreceptor retinoid-binding protein (IRBP) by Y-79 human retinoblastoma cells, *J. Cell. Physiol.*, 124 (1985) 233–239.

[19] Mezei, C. and Wainwright, S.D., Hormone-induced increase of hydroxyindole-*O*-methyltransferase activity in the embryonic chick pineal gland in organ culture, *Life Sci.*, 24 (1979) 1111–1118.

[20] Parfitt, A., Weller, J.L. and Klein, D.C., Beta adrenergic blockers decrease adrenergically stimulated N-acetyltransferase activity in pineal glands in organ culture, *Neuropharmacology*, 15 (1976) 353–358.

[21] Pierce, M.E., Barker, D., Harrington, J. and Takahashi, J., Cyclic AMP-dependent melatonin production in Y-79 human retinoblastoma cells, *J. Neurochem.*, 53 (1989) 307–310.

[22] Quay, W.B., Retinal and pineal hydroxyindole-*O*-methyltransferase activity in vertebrates, *Life Sci.*, 4 (1965) 983–991.

[23] Rodriguez, I.R., Mazuruk, K., Schoen, T.J. and Chader, J.G., Structural analysis of the human hydroxyindole-*O*-methyltransferase gene: presence of two distinct promoters, *J. Biol. Chem.*, 269 (1994) 31969–31977.

[24] Samuels, H.H., Stanley, F. and Casanova, J., Depletion of L-3,5,3′-triiodothyronine and L-thyroxine in euthyroid calf serum for use in cell culture studies of the action of thyroid hormone, *Endocrinology*, 105 (1979) 80–85.

[25] Smith, P.J., Wise, L.S., Berkowitz, R. Wan, C. and Rubin, C.S., Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes, *J. Biol. Chem.*, 263 (1988) 9402–9408.

[26] Sugden, D. and Klein, D.C., Regulation of rat pineal hydroxyindole-*O*-methyltransferase in neonatal and adult rats, *J. Neurochem.*, 40 (1983) 1647–1653.

[27] Sugden, D. and Klein, D.C., β-Adrenergic receptor control of rat pineal hydroxyindole-*O*-methyltransferase, *Endocrinology*, 113 (1983) 348–353.

[28] Sugden, D., Ceña, V. and Klein, D.C., Hydroxyindole-*O*-methyltransferase, *Methods Enzymol.*, 142 (1986) 590–596.

[29] Voisin, P., Guerlotté, J. and Collin, J.-P., An antiserum against chicken hydroxyindole-*O*-methyltrasferase reacts with the enzyme from pineal gland and retina and labels pineal modified photoreceptors, *Mol. Brain Res.*, 4 (1988) 53–61.

[30] Wurtman, R.J., Axelrod, J. and Fischer, J.E., Melatonin synthesis in the pineal gland: effect of light mediated by the sympathetic nervous system, *Science*, 143 (1964) 1328.