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Retinoic Acids Induce Neurosteroid Biosynthesis in Human Glial GI-1 Cells via the Induction of Steroidogenic Genes

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The steroids synthesized in the central nervous system (CNS) are the neurosteroids. Since little information is currently available concerning the roles of the retinoic acids (RAs) during steroidogenesis in the CNS, we investigated the effects of RAs upon their synthesis in our current study. Specifically, we analyzed the effects of all-*trans*-retinoic acid (ATRA) upon the expression of neurosteroid biosynthesis genes in the human glial cell line GI-1, in which the major steroidogenic genes are expressed. Treatment with ATRA (10 μM) induced a 4.9-fold increase in the expression of the cytochrome P450scc (CYP11A1) gene, the product of which cleaves the cholesterol side chain, a rate-limiting step during steroidogenesis. ATRA also strongly induced the expression of steroidogenic acute regulatory protein (StAR) and 3β-hydroxysteroid dehydrogenase (3β-HSD) (an increase of 5- and 50-fold, respectively). A retinoic acid receptor (RAR)-specific agonist, TTNPB, was unable to mimic this induction whereas a retinoid X receptor (RXR)-specific agonist, methoprene acid, in addition to 9-*cis*-RA, could do so. These data indicate that ATRA is isomerized to 9-*cis*-RA in the culture medium, as reported previously, and that 9-*cis*-RA activates the RXR. In addition, ATRA also induced the de novo synthesis of neurosteroids such as pregnenolone and progesterone. These results suggest that ATRA might induce the *de novo* neurosteroid synthesis via the induction of steroidogenic genes in human glial cells. The multiple effects of vitamin A upon CNS functions might therefore be partly explained by the induction of neurosteroidogenesis by RAs, since neurosteroids have also been reported to have multiple effects in the CNS.

Key words: CYP11A1, glial cells, neurosteroid, retinoic acid, steroidogenesis.

Abbreviations: ATRA, all-*trans*-retinoic acid; CHOL, cholesterol; CNS, central nervous system; CYP, cytochrome P450; DMEM, Dulbecco's modified Eagle's medium; DHEA, dehydroepiandrosterone; $GABA_A$, γ-aminobutyric acid A; 3β-HSD, 3β-hydroxysteroid dehydrogenase; NMDA, *N*-methyl-*D*-aspartate; PBR, peripheral-type benzodiazepine receptor; PREG, pregnenolone; PROG, progesterone; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; StAR, steroidogenic acute regulatory protein; TLC, thin layer chromatography; TOSP, translocator protein; TTNPB, 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthalenyl)-propenyl]benzoic acid.

The nervous system is an important site of steroid production, within which both neurons and glial cells can synthesize these molecules *de novo* from cholesterol. Steroids that are synthesized within the central or peripheral nervous systems, have been designated the 'neurosteroids' (*1–4*). The mitochondrial cytochrome P450scc (CYP11A1), the cholesterol side chain cleavage enzyme which catalyzes the *de novo* synthesis of pregnenolone (PREG), is expressed throughout the rodent brain (*5–7*). Immunochemical and biochemical studies have also demonstrated that very low amounts of CYP11A1 mRNA are regionally expressed (*8*). Kimoto *et al*. have demonstrated in their previous study that the CYP11A1 protein localizes in the rat hippocampal neurons (*9*). The 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme, which converts PREG to progesterone (PROG), is also largely distributed throughout the brain and spinal cord (*10*,*11*). In addition, primary cultures of mixed glial cells can metabolize cholesterol to PREG and PROG (*12*, *13*). However, the molecular basis of the regulation of neurosteroid synthesis remains unclear.

The retinoic acids (RAs) are vitamin A derivatives and form part of a complex signalling system that is essential for normal development and homeostasis in vertebrates (*14*). These molecules also exert a variety of biological actions that are mediated via the expression of specific target genes. RAs have further been reported to regulate steroid biosynthesis in steroidogenic tissues such as the adrenal gland (*15*), ovary (*16*) and testis (*17–19*). However, little information is currently available concerning the roles of the RAs in the regulation of steroidogenesis in the central nervous system (CNS), although our understanding of the physiological role of retinoid signalling in the CNS is increasing (*20–22*). Retinoid signalling has been implicated in a variety of nervous

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system disorders such as schizophrenia (*23*) and depression (*24*,*25*). Dysfunctional retinoid signalling can also trigger cognitive impairment (*26*). Neurosteroids are also thought to be involved in many kinds of CNS disorders, including depression (*27*), attention deficit and hyperactivity (*28*), post-traumatic stress (*29*) and panic (*30*). It is thus tempting to speculate that the effects of the RAs on the CNS are partly due to alterations in the neurosteroid biosynthesis process in neural cells. To elucidate the possible effect of RAs on the *de novo* neurosteroid synthesis in the neural cells, we have in our current study analyzed the effect of all-*trans* retinoic acid (ATRA) on the expression neurosteroid biosynthesis genes and on the production of neurosteroids in human glial cells.

MATERIALS AND METHODS

Reagents—ATRA and 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-napthalenyl)-1-propenyl]benzoic acid (TTNPB) were purchased from Wako Pure Chemicals (Tokyo, Japan). Cycloheximide was purchased from Nacalai (Tokyo, Japan). DHEA, 9-*cis*-retinoic acid, PREG and PROG were obtained from Sigma (St. Louis, MO). Methoprene acid was purchased from Biomol International (Plymouth, PA).

Cell Culture—GI-1 is a human glial cell line established from a tumour specimen removed from the left frontparietal region of a 61-year-old man and was obtained from the Riken Cell Bank (Tokyo, Japan). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 U/ml penicillin and 10 U/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2. For RA treatments, exponentially growing cells were split into six-well plates (3×10^5 cells/well) and cultured for 7 days in medium supplemented with 10% FBS. This growth medium was exchanged for DMEM containing 5% charcoal-treated serum and RAs at various concentrations.

Reverse transcriptase PCR—Total RNAs were isolated using the guanidium thiocyanate phenol-chloroform extraction method (*31*). First strand cDNA was synthesized from 5 μg of total RNA using 100 units of reverse transcriptase (ReverTra Ace, TOYOBO, Tokyo) and with random primers, according to the manufacturer's protocol. PCR was then carried out, using this synthesized cDNA as a template, with Taq polymerase (GoTaq, Promega, Madison, WI). The amplification conditions were as follows: 1 min at 95°C, 1 min at 58°C and 1 min at 72°C. Quantitative real-time PCR was performed using an ABI-Prism 7300 thermal cycler and a SYBR green PCR reagent kit (Roche Diagnostics K. K., Tokyo, Japan). To measure CYP11A1 gene expression, a hydrolysis probe for this gene (Universal Probe Library #59, Roche) was used. Samples were denatured at 94°C for 10 min, and cDNA products were amplified with 40 cycles of denaturation at 95°C for 15 s, then annealing and extension at 60°C for 60 s. Calculations of the initial amounts of mRNA were performed according to the cycle threshold method (*32*). The mRNA levels were normalized using the 18S rRNA levels, which had been quantified by real-time PCR. The PCR primers used to amplify the steroidogenic cDNAs were designed from published DNA sequences using Primer Express *ver*. 3.0. The sequences of the primers used are as follows; 18S rRNA, forward 5′-TGG TTG CAA AGC TGA AAC TTA AAG-3′ and reverse 5′-AGT CAA ATT AAG CCG CAG GC-3′; PBR, forward 5′-TGG GCT CCC GCT TTG TC-3′ and reverse 5′-GTC GGG CAC CAA AGA AGA TG-3′; StAR, forward 5′-CCA CCC CTA GCA CGT GGA T-3′ and reverse 5′-ATT GTC CTG CTG ACT CTC CTT CTT-3′; CYP11A1, forward 5′-CCA GTA TTA CCA GAG ACC CAT AGG A-3′ and reverse 5′-TTA GTG ATG GAC TCA AAG GCA AAG-3′; HSD3B1, forward 5′-GCC AGG ACG TCT CGG TCA T-3′ and reverse 5′-CTT TTT GCT GTG TGG GTA TGG A-3′; CYP17A1, forward 5′-TAC AAG GAG AAA TTC CGG AGT GA-3′ and reverse 5′-TGC CAC TCC TTC TCA TTG TGA T-3′; SF-1, forward 5′-ACC AGA CCT TCA TCT CCA TCG T-3′ and reverse 5′-CGT CTT TCA CCA GGA TGT GGT T-3′; RARα, forward 5′-CCA AGG AGT CTG TGA GAA ACG A-3′ and reverse 5′-GAG ACA CGT TGT TCT GAG CTG TTG-3′; RARβ, forward 5′-TCT CAG TGC CAT CTG CTT AAT CTG-3′ and reverse 5′-CTG CAC CTT TAG CAC TGA TGC T-3′; RARγ, forward 5′-GCA TTG CTG ACC AGA TCA CTC T-3′ and reverse 5′-CCC AGC AAA GGC AAA GAC AA-3′; RXRα, forward 5′-GAC CCT GTC ACC AAC ATT TGC-3′ and reverse 5′-CGT CAG CAC CCT GTC AAA GAT-3′; RXRβ, forward 5′-GTG TCC AAA ATG CGT GAC ATG-3′ and reverse 5′-GAG GGC AGG AAG ACG TAG CA-3′; RXRγ, forward 5′-GGG TCG GCT CCA TCT TTG A-3′ and reverse 5′-GTT CCG GAT ACT TCT GCT TGG T-3′.

Radiolabelling of Steroid Hormones and Thin Layer Chromatography—To label cholesterol and steroid molecules, 1 mCi/ml of (1-^{14}C)acetic acid sodium salt (57 mCi/mmol, Moravek Biochemicals, Brea, CA) was added to the culture for 24 h. The culture medium was then collected and steroids were extracted as described previously (*33*). Briefly, an equal volume of ethyl acetate : isooctane (1 : 1 v/v) was added to the media. This mixture was then centrifuged and the upper organic phase was collected. The extraction procedure was repeated twice and the organic phase was dried and resuspended in 20 μl ethyl acetate. The steroids (5 μl) were separated on silica-gel 60 F_{245} plates (Merck, Darmstadt, Germany) with chloroform : ethyl acetate (4 : 1) as the mobile phase. The radioactive lipids on the thin layer chromatography (TLC) plate were visualized using an FLA7000 device (Fuji Film, Tokyo, Japan). A non-radioactive standard mixture was included on the plate and visualized by UV or phosphomolybdic acid. The migration of standard steroids was used to identify the radioactive steroids on the same plate.

Statistical Analysis—Data were statistically analyzed using Dunnett's multiple comparison tests. Results were considered significant when $P < 0.05$.

RESULTS

Expression of Steroidogenic Genes in the GI-1 Human Glial Cell Line—During the process of steroid biosynthesis, cholesterol is transported across the outer and to the

inner mitochondrial membrane through a complex containing the peripheral-type benzodiazepine receptor (PBR), recently renamed as translocator protein (TOSP) (*34–36*) and the steroidogenic acute regulatory protein (StAR) (*37*). In the inner membrane, CYP11A1 converts cholesterol to PREG (3β-hydroxypren-5-en-20-one), and 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) then converts PREG to PROG. PREG is also converted to dehydroepiandrosterone (DHEA) by cytochrome P450 17α-hydroxylase and C17,20 lyase, which are encoded by a single gene, CYP17A1. To determine, whether the human glial GI-1 cells, a human glial cell line established from a tumour specimen removed from the left frontparietal region of a 61-year-old man, express these steroidogenic and related enzyme genes, we analyzed the PBR, StAR, CYP11A1, 3β-HSD and CYP17A1 genes by RT-PCR. As shown in Fig. 1A, GI-1 cells express all of these tested genes.

Effects of ATRA on Steroidogenic Gene Expression in GI-1 Cells—To investigate the effect of retinoids upon neurosteroid biosynthesis, we analyzed the effects of ATRA on the expression of steroidogenic genes in GI-1 cells. As shown in Fig. 2A, ATRA induces the expression of the StAR, CYP11A1 and 3β-HSD genes by 5.5-, 4.9- and 50-fold, respectively, at a concentration of 10 μM ATRA after 48 h of incubation. This induction was found to occur in a dose-dependent manner. The expression of the CYP11A1 gene increased almost linearly up to 48 h in the presence of 1 μM ATRA as shown in Fig. 2B. On the other hand, the expression of the CYP17A1 and SULT2B1 genes did not change (data not shown).

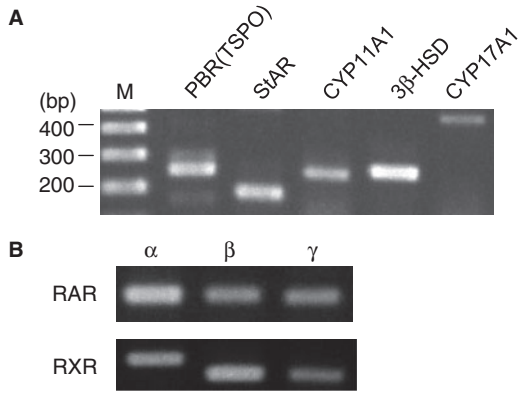

Fig. 1. **The expression of steroidogenic and retinoid receptor genes in the human glial GI-1 cell line.** (A) The indicated steroidogenic genes were analyzed by RT-PCR in GI-1 cells. M, size markers. (B) RAR and RXR expression in GI-1 cells was analysed by RT-PCR. Subtype-specific primers for α, β and γ were used.

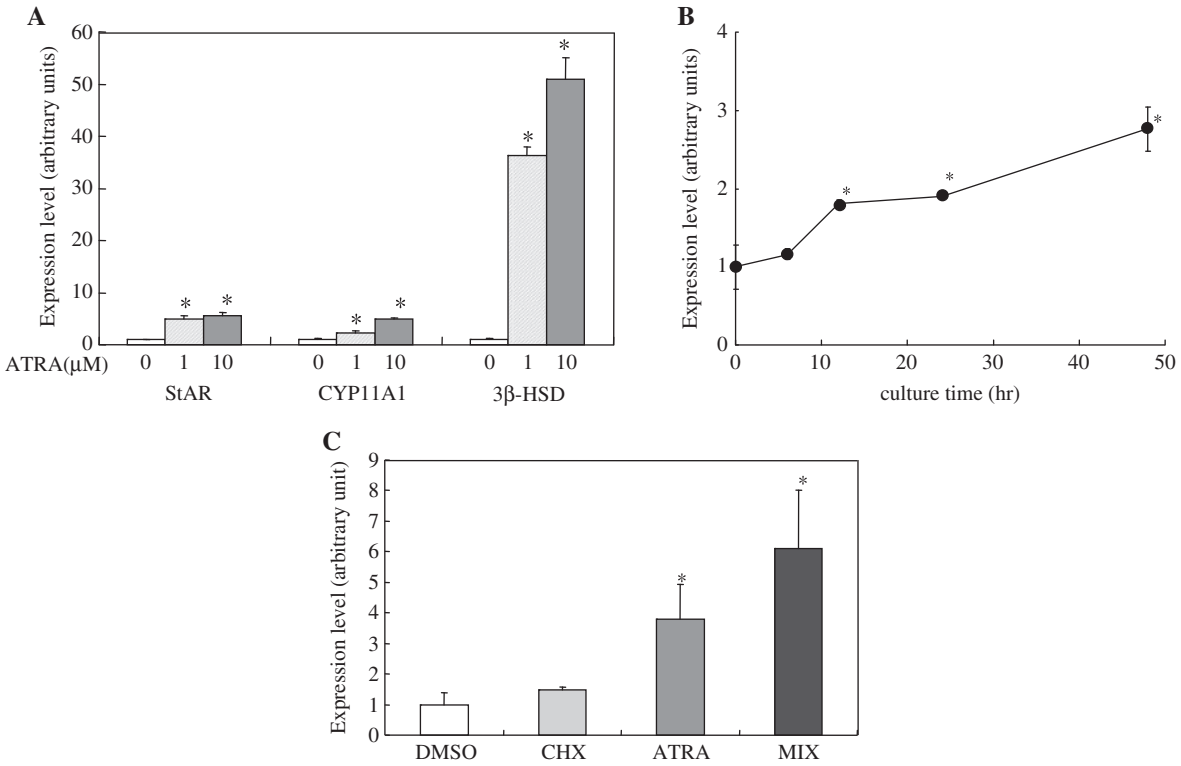

Fig. 2. **ATRA induces steroidogenic genes in GI-1 cells.** (A) GI-1 cells were treated with ATRA (at 0, 1 and 10 μM) for 48 h. Total RNA was then isolated followed by quantitative RT-PCR analysis of StAR, CYP11A1 and 3β-HSD. The results are presented using arbitrary units, with the control values set at 1, and are the means ± SD of three experiments. Open bars, ATRA = 0 μM; hatched bars, ATRA = 1 μM; closed bars, ATRA = 10 μM. $^*P < 0.05$. (B) GI-1 cells were incubated with 1 μM ATRA for 48 h. At the indicated times, cells were harvested and subjected to real-time PCR analysis of CYP11A1 ($n = 3$). $^*P < 0.05$. (C) GI-1 cells were treated with 10 μM ATRA (ATRA), 10 μM cycloheximide (CHX) or both (MIX) for 48 h. CYP11A1 was analyzed by quantitative RT-PCR. The results are presented using arbitrary units, with the control values (DMSO) set at 1, and are the means ± SD of three experiments. $^*P < 0.05$.

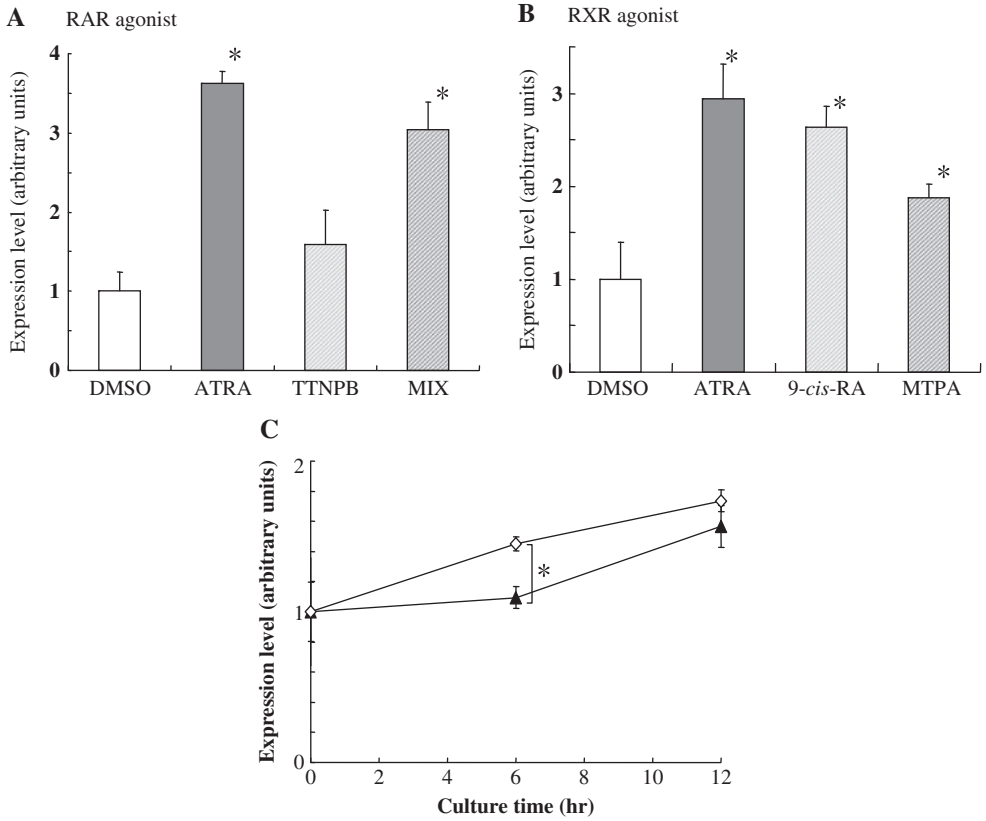

Fig. 3. **Effects of retinoid receptor agonists upon CYP11A1 gene expression in GI-1 cells.** (A) GI-1 cells were treated with ATRA (10 μM), RAR-specific agonist TTNPB (10 μM) or both for 48 h. CYP11A1 was then analyzed in these cells by quantitative RT-PCR. The values shown are the means ± SD ($n = 3$). (B) Effects of the RXR-specific agonists methopene acid (MTPA) (100 μM) and 9-*cis*-RA (10 μM), which transactivates both RAR and RXR, upon CYP11A1 gene expression measured by quantitative RT-PCR as described in (A). The values shown are the means ± SD ($n = 3$). $^{*}P < 0.05$. (C) Induction of CYP11A1 expression by 9-*cis*-RA (10 μM) and ATRA (10 μM) at the early stage of culture. 9-*cis*-RA, open diamond; ATRA, closed triangle. The values shown are the means ± SD ($n = 3$). $^{*}P < 0.05$.

Moreover, the induction of CYP11A1 was not inhibited by cycloheximide (Fig. 2C), showing that *de novo* protein synthesis was not necessary for the induction. Furthermore, no obvious morphological changes had occurred as a result of ATRA treatment after 48 h (data not shown).

Effects of Retinoid Receptor Agonists on the CYP11A1 Gene Expression in GI-1 Cells—It has been well established that the actions of the RAs are mediated by their binding to cognate nuclear receptors (RARs), which then form heterodimers with RXRs and bind to RA responsive elements (RAREs) located in the regulatory regions of specific target genes (*38*). In our current analyses, we detected the expression of three RAR isoforms (RARα, RARβ and RARγ) in GI-1 cells (Fig. 1B). To investigate the involvement of the RARs in the induction of CYP11A1 genes by ATRA, we examined the effects of TTNPB, an agonist for three RAR isoforms, but found that it does not activate CYP11A1 expression in GI-1 cells (Fig. 3A). It has been reported that retinoic acids undergo a thiol radical-mediated isomerization in cell culture medium resulting in a mixture of 9-*cis*-RA, 13-*cis*-RA and ATRA molecules (*39*). Since three RXR isoform genes are expressed in GI-1 cells (Fig. 1B), we next examined the effects of 9-*cis*-RA and methoprene acid, a RXR specific agonist (*40*). As shown in Fig. 3B, both of these molecules significantly induced the expression of CYP11A1 as potently as ATRA. Moreover, 9-*cis*-RA was able to induce CYP11A1 expression more rapidly (within 6 h) than ATRA (12 h) at the same concentrations as shown in Fig. 3C.

ATRA Treatment Enhances Endogenous Neurosteroid Production in GI-1 Cells—To demonstrate that the steroidogenic enzymes were active and functional in GI-1 cells, cells were treated with ATRA for 48 h and then [1-^{14}C]acetic acid was added to the culture medium for 24 h prior to collecting the medium. In this experiment, the radioactive acetate molecules will be incorporated into mevalonate and then cholesterol, and thereby enter the steroid biosynthetic pathway. Radiolabelled steroids were extracted from the culture media samples, separated by TLC and visualized. As shown in Fig. 4A, low but significant levels of PREG and PROG were synthesized and secreted into the culture medium in addition to cholesterol. Following the addition of ATRA, the *de novo* synthesis of PROG and PREG increased, whereas that of cholesterol was reduced (Fig. 4A). An unidentified spot above PROG (designated by asterisk) was also visible as reported by Zhu and

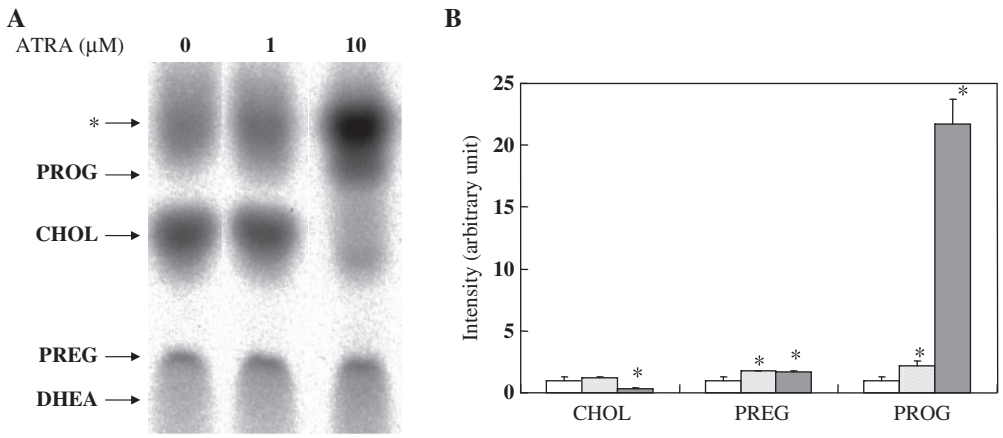

Fig. 4. **Induction of neurosteroid synthesis in GI-1 cells by exposure to ATRA.** (A): GI-1 cells were treated with ATRA (0, 1 and 10 μM) for 48 h. The ^{14}C-labelled steroids in the culture medium were extracted with ethyl acetate:isooctane (1 : 1 v/v) and separated by TLC. The radioactivity on the TLC plate was visualized using a FLA7000. Arrows indicate the positions of the steroid standards. PREG, pregnenolone; PROG, progesterone; CHOL, cholesterol and DHEA, dehydroepiansrosterone. The asterisk indicates an unidentified compound as reported by Zhu and Glaser (*33*), the synthesis of which also appears to be accelerated by ATRA. (B) The intensities of the spots corresponding to the CHOL, PREG and PROG were quantified with an image analyzer. Open bars, ATRA = 0 μM; hatched bars, ATRA = 1 μM; closed bars, ATRA = 10 μM. The results are presented using arbitrary units, with the control values set at 1. The values are the means ± SD of three independent labelling experiments. $^{*}P < 0.05$.

Glaser (*33*), and its synthesis was also induced by ATRA (Fig. 4A). From the band intensities, the levels of production of steroids were quantified, and the induction indexes were calculated to be 1.7 and 22 for PREG and PROG, respectively, at 10 μM ATRA (Fig. 4B). These results confirmed that the steroidogenic enzymes were indeed functionally active and that ATRA induces the *de novo* synthesis of PREG and PROG via the upregulation of steroidogenic genes in GI-1 cells. The increase in the *de novo* synthesis of neurosteroids was almost parallel with the expression levels of the corresponding genes induced by ATRA.

DISCUSSION

A large body of evidence has been produced concerning the role of retinoids, especially ATRA, in CNS development (*21*, *22*, *41*). There is a dearth of information available, however, regarding the effects of ATRA upon the regulation of neurosteroid biosynthesis. In our current study, we have demonstrated that ATRA strongly induces steroidogenic gene expression and consequently stimulates neurosteroid production in the human glial GI-1 cell line.

Since the glia represents a primary site for neurosteroidogenesis (*42*), we utilized the human glial cell line, GI-1, to investigate the effects of the RAs upon neurosteroid production. By RT-PCR we revealed that GI-1 cells express the steroidogenic genes that are required for the production of PREG, DHEA and PROG at least (Fig. 1).

RAs have been reported to regulate steroid biosynthesis in a number of steroidogenic tissues such as the adrenal gland (*15*), ovary (*16*) and testis (*17–19*). We demonstrated in our current study for the first time that RAs also regulate steroid biosynthesis in human neural cells. ATRA was found to induce a set of steroidogenic genes including StAR, CYP11A1 and 3β-HSD in the human glial GI-1 cell line (Fig. 1A). Since CYP11A1 is the first and also rate-limiting cholesterol side chain cleavage enzyme, we further investigated the induction of this gene by ATRA. Using subtype-specific agonists (TTNPB for RAR and methoprene acid for RXR) we found that the induction of CYP11A1 is mediated by RXR instead of RAR. In this regard, ATRA is known to only transactivate RAR whereas 9-*cis*-RA binds to both types of RA receptors. We thus speculate that 9-*cis*-RA, which is likely to be produced by the isomerization of ATRA in the GI-1 culture medium as described by Lanvers *et al*. (*39*), might transactivate RXR and thereby induces CYP11A1. This is supported by the result of Fig. 4C; 9-*cis*-RA was more effective to induce CYP11A1 expression than ATRA.

Several putative consensus retinoic acid response elements have been found in the promoter of the CYP11A1 gene (*43*), its regulation by ATRA or 9-*cis*-RA may result from the direct activation of its promoter by RAR and RXR receptors. This is also supported by our observation that cycloheximide does not inhibit the induction of this gene by ATRA (Fig. 2C).

We did not further investigate the induction of the StAR and 3β-HSD genes by ATRA. Because several putative consensus retinoic acid response elements have been identified previously within the promoters of these genes (*43*), ATRA or 9-*cis* RA might also directly induce the expression of these genes. Consistently, the RAs have been reported to induce StAR expression in K28 mouse Leydig and adrenal tumour cells, Y1 (*44*). However, it has also been reported that the RAs do not induce CYP11A1 or 3β-HSD gene expression in K9 mouse Leydig tumour cells (*45*). We could not detect any expression of nuclear protein steroidogenic factor 1 (SF-1),

which plays a central role in regulating steroidogenic genes in the classical endocrine tissues (data not shown). The lack of SF-1 expression in rat glial cells has also been reported (*46*). The evidence to date thus suggests that in neural cells, RAs regulate the expression of steroidogenic genes in a manner that differs from classical steroidogenic tissues. An investigation of the molecular basis of the ATRA-induced expression of StAR and 3β-HSD in GI-1 cells, is currently underway in our laboratory.

We also demonstrate in our current study that neurosteroids are produced in GI-1 cells by labelling steroids with [1-^{14}C]acetic acid (Fig. 4). These assays confirmed that the steroidogenic enzymes are functionally active in GI-1 cells. The induction of steroidogenic genes by ATRA resulted in elevated levels of radiolabelled neurosteroids in the GI-1 cells, indicating increased enzyme activities. At 1 μM ATRA, the increase in PROG production was only 2-fold although the gene expression of 3βHSD, which converts PREG to PROG, was elevated 36-fold. This indicates that the rate-limiting step for steroidogenesis in GI-1 cells is the side-chain cleavage reaction catalyzed by CYP11A1, the expression, of which was found to be induced 2.2-fold by 1 μM ATRA. This is similar to the situation in the classical steroidogenic tissues. The enhanced level of PROG production (22-fold) was almost proportional to the induction index of the 3β-HSD gene (51-fold) at 10 μM ATRA, at which CYP11A1 expression was increased 4.9-fold. Interestingly, the production levels of PREG at 10 μM ATRA were equivalent to those at 1 μM. This is probably due to the elevated metabolism of PREG to PROG catalyzed by highly activated 3β-HSD. It should be pointed out that the radiolabelled steroids in the culture medium in the current study do not represent all *de novo* synthesized steroids. The transportation of neurosteroids across the cell membrane may be another determinant of the concentrations of neurosteroids in the culture medium, but this remains uncertain at present.

In conclusion, we show in our current report for the first time that RA induces neurosteroid production in human glial cells in culture. Evidence has accumulated to date showing that retinoids affect adult brain function, particularly learning and memory, locomotor activity and depression (*22*, *47*). However, the molecular basis for the effect of RAs has not been well documented. Neurosteroids have also been reported to affect multiple brain functions (i.e. neuroendocrine and behavioural functions). In addition to the classical genomic actions of steroids, neurosteroids can rapidly alter the excitability of the CNS through their binding to neurotransmitter-gated ion channels, thus modulating the γ-aminobutyric acid A ($GABA_A$) and *N*-methyl-*D*-aspartate (NMDA) receptors. These overlapped effects of the RAs and neurosteroids on brain functions lead us to hypothesize that RAs exert their effects on the CNS by modulating neurosteroid biosynthesis in neural cells. Our finding of ATRA-induced neurosteroid biosynthesis in glial cells will provide a clue to our understanding of the multiple effects of RAs on the functions of the CNS. Further experiments using isolated primary cultured glial cells should be performed to evaluate our hypothesis.

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CONFLICT OF INTEREST

None declared.

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