

Neural Adrenergic/Cyclic AMP Regulation of the Immunoglobulin E Receptor α -Subunit Expression in the Mammalian Pinealocyte

A NEUROENDOCRINE/IMMUNE RESPONSE LINK?^{*[5]}

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The high affinity immunoglobulin E receptor (Fc ϵ RI) complex is dedicated to immunoglobulin E-mediated allergic responses. Expression of the Fc ϵ RI receptor is thought to be relatively stable and limited to mast cells, basophils, eosinophils, monocytes, Langerhans cells, platelets, and neutrophils. We now report that the Fc ϵ RI α and Fc ϵ RI γ polypeptides are expressed in the pinealocyte, the melatonin-secreting cell of the pineal gland. Moreover, *Fcer1a* mRNA levels increased ~100-fold at night to levels that were higher than in other tissues examined. Pineal Fc ϵ RI α protein also increased markedly at night from nearly undetectable daytime levels. Our studies indicate that pineal *Fcer1a* mRNA levels are controlled by a well described neural pathway that controls pineal function. This pathway includes the master circadian oscillator in the suprachiasmatic nucleus and passes through central and peripheral structures. The circadian expression of Fc ϵ RI α in the pineal gland is driven by this neural circuit via an adrenergic/cyclic AMP mechanism. Pineal Fc ϵ RI α and Fc ϵ RI γ may represent a previously unrealized molecular link between the neuroendocrine and immune systems.

A resonant theme in the initiation of an allergic reaction is the binding of allergens to immunoglobulin E (IgE) antibodies

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attached to their corresponding high affinity Fc receptors (Fc ϵ RI)³ on mast cells or basophils. This event is essential for a broad range of events including immediate induction of signaling cascades leading to degranulation, which releases stored mediators including histamine, serotonin, and proteases, and increases the production of arachidonic acid. This results in the generation of symptoms characteristic of acute allergic reaction (1–4). The subsequent late phase reaction is associated with secretion of immunoregulatory cytokines and chemokines leading to an influx of T-cells, monocytes, and eosinophils to the target tissue (5, 6).

Fc ϵ RI is a multimeric receptor composed of α -chain, β -chain, and γ -homodimer ($\alpha\beta\gamma_2$) or α -chain and γ -homodimer ($\alpha\gamma_2$) (4, 6). The extracellular portion of α -subunit binds IgE molecules via the Fc domain. The β - and γ -subunits are not involved directly in IgE binding but are essential for membrane localization and signal transduction in that domains of both subunits are exposed to the cytoplasm (7). Signaling is triggered by the simultaneous binding of a single multivalent allergen to multiple IgE·FcR complexes leading to immunogen-induced cross-linking of the receptor on the cell surface. Tetrameric ($\alpha\beta\gamma_2$) and trimeric ($\alpha\gamma_2$) complexes are known to be active (7).

Expression of Fc ϵ RI was originally thought to be limited to the effector cells of anaphylaxis, specifically mast cells and basophils. Later, it became apparent that the Fc ϵ RI is expressed in antigen-presenting cells including human epidermal Langerhans cells, peripheral blood dendritic cells, monocytes, and eosinophils (7, 8). Expression of Fc ϵ RI has not been reported in neural, endocrine, or neuroendocrine cells.

The results presented in this report reflect a surprising observation made during the course of a preliminary microarray

³ The abbreviations used are: Fc ϵ RI, high affinity immunoglobulin E receptor; NE, norepinephrine; ISO, isoproterenol; ZT, zeitgeber time; SCG, superior cervical ganglion; RBL, rat basophilic leukemia; SCN, suprachiasmatic nucleus; PBS, phosphate-buffered saline; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.

analysis of gene expression in the pineal gland, a tissue not known to be involved in the immune response. This tissue is recognized as being dedicated to the generation of the daily rhythm in circulating melatonin, a conserved feature of vertebrate biology (9). Our microarray results were surprising because they suggested that there is a daily rhythm in *Fcer1a* expression in the pineal gland and that nighttime levels are higher than in other tissues. This was especially intriguing when viewed against the evidence of highly restricted expression of Fc ϵ RI and our current concept of the pineal gland as a melatonin factory. The potential importance of this discovery propelled us to confirm the preliminary finding of a 24-hour rhythm in *Fcer1a* mRNA and to extend this with the intention of obtaining a better understanding of Fc ϵ RI biology in the pineal gland. Our findings provide justification to consider that Fc ϵ RI might provide a unique molecular link between the neuroendocrine and immune systems.

EXPERIMENTAL PROCEDURES

Animals and Tissue Removal—Sprague-Dawley rats (Taconic Farms Inc., Germantown, NY) were used for all experiments. Animals were housed in a controlled lighting environment (14-h light and 10-h dark cycles, and for *in situ* hybridization studies light/dark 12:12). In experiments presented in Fig. 5A, animals were kept under constant darkness or constant light for 3 days. Surgical decentralization of the superior cervical ganglia (SCGdcn, Taconic Farms Inc.) was performed. Rats were allowed to recover and adjust to the light cycle for 2 weeks and then were sacrificed by CO₂ asphyxiation and decapitated at the indicated zeitgeber time (ZT). During the dark period, animals were handled under red photographic safelights. Pineal glands and other tissues were removed immediately and frozen on dry ice. All procedures were done in accordance with National Institutes of Health animal use and care guidelines. For *in situ* hybridization studies, Wistar rats were used. Animals (Animal Section, Panum Institute, Copenhagen, Denmark) were housed for 2 weeks in a controlled lighting environment (12-h light and 12-h dark cycles). As indicated in Fig. 6A, animals were injected intraperitoneally with isoproterenol (ISO, 10 mg/ml phosphate-buffered saline, 5 mg/kg rat).

Cell and Organ Culture—Pinealocytes were prepared from rat pineal glands (10). Briefly, pineal glands were incubated (1 h, 37 °C) with 20 units/ml papain (Worthington) and 200 units/ml DNase I (Worthington) in Earle's balanced salt solution. Subsequently, glands were triturated, and the resulting preparation was passed through a 40- μ m cell strainer (BD Falcon, Bedford, MA). The pinealocytes were harvested, washed, and suspended in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μ g/ml streptomycin (95% air, 5% CO₂, 37 °C). Dispersed pinealocytes were cultured either in suspension (for Western blot analysis in Fig. 6C) or as an adherent monolayer (for immunocytochemical detection in Fig. 4) on slides pre-coated with polyornithine (Sigma). About 16 h following the cell preparation, the pinealocytes were treated with norepinephrine (NE, 1 μ M), dibutyryl cyclic AMP (1 mM), or forskolin (10 μ M) for 6 h.

The rat basophilic leukemia (RBL) cell line RBL-2H3 (ATCC, Manassas, VA) (11) was grown as an adherent monolayer in tissue culture flasks using Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum in 5% CO₂ incubator at 37 °C. Pineal glands were cultured as described (Ref. 12 and supplemental data). Bone marrow extraction from 2–3-month-old mice and cell preparations were done according to published procedures (Ref. 13 and supplemental data).

Immunochemical Reagents—The α -subunit of Fc ϵ RI was detected using mouse monoclonal antibody ER that was raised against the α -subunit of Fc ϵ RI expressed in RBL-2H3 cells (11). S-antigen/arrestin was detected using a polyclonal rabbit anti-bovine S-antigen antiserum (NEI Z-02 from Dr. S. Zigler, NEI, National Institutes of Health). Mouse monoclonal anti-synaptophysin (Santa Cruz Biotechnology) and rabbit polyclonal anti-Fc ϵ RI γ were purchased (Upstate Biotechnology).

Immunocytochemistry and Fluorescence Microscopy—All immunocytochemical analyses (see Fig. 4) were done with pinealocytes attached on multichambered slides (Sigma). Dispersed pinealocytes (1 \times 10⁶ cells/chamber) were transferred to polyornithine-coated chambers on the slide. Cells were allowed to attach during overnight incubation (95% O₂, 5% CO₂, 37 °C). On the following day, the cells were treated with NE (1 μ M) for 6 h, washed twice in phosphate-buffered saline (PBS, pH 7.4), fixed (2% paraformaldehyde, 20 min, 24 °C), and permeabilized (0.05% saponin, 30 min, 24 °C). The permeabilized cells were rinsed with PBS and then in PBS containing 0.1 M glycine and blocked with 2% bovine serum albumin in PBS containing 5 μ g/ml donkey IgG. The cells were then rinsed twice with PBS and incubated with primary antibodies. After incubation with primary antibodies against α - and γ -subunits of the Fc ϵ RI receptor (10 and 5 μ g/ml, respectively), cells were rinsed thoroughly in PBS and incubated with goat anti-mouse F(ab')₂ conjugated to Alexa 488 (for α -subunit) or goat anti-rabbit F(ab')₂ conjugated to Alexa 594 for γ -subunit (Molecular Probes, Eugene, OR). Finally, cells were rinsed in PBS and then in distilled water. Coverslips were mounted with Fluoromount G (EM Sciences, Fort Washington, PA). For negative controls, cells were treated identically and incubated only with secondary antibodies omitting primary antibodies.

Images were acquired by scanning confocal microscopy (Leica TCS NT, Heidelberg, Germany). The confocal parameters were established at the beginning of the study and remained constant throughout with an equal brightness setting and a constant confocal aperture pinhole optimized for the objective lens used.

In Situ Hybridization and Northern Blot—These methods were done using published techniques (14, 15) as detailed in supplemental data. For *in situ* hybridization, two 38-mer oligonucleotide probes, IgE rec-1 and IgE rec-2 (derived from GenBankTM accession number M21622 antisense sequence), were used: 5'-TTG TTC AAA TAG CCT GTG CAG TGG TAG CTG CCA CTG TC-3' (IgE rec-1) and 5'-TTG TCT AAG ACC ACG TCA GCA GAA GAT TGG AGC AGC AG-3' (IgE rec-2). For Northern blot analysis, probes described previously (14) were used that were generated from the *Fcer1a* coding region nucleotides 177–600 (GenBankTM accession number M21622), *Fcer1b* coding nucleotides 2051–2470 (GenBankTM

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accession number M22923), and *Fcer1g* coding nucleotides 61–421 (GenBankTM accession number L04306), respectively.

Real-time PCR and Quantification—RNA isolation, RNA processing, quantification, and cDNA synthesis were done as described previously (Ref. 15 and supplemental data). Real-time quantitative PCR was performed using a LightCyclerTM rapid thermal cycler system (Roche) (Refs. 16, 17 and supplemental data). Primers used in the analysis are as follows: rat β -actin (V01217) 5'-ACG GTC AGG TCA TCA CTA TGG-3' (sense); and 5'-AGC CAC CAA TCC A CA CAG A-3' (antisense); rat *Aanat* (U38306) 5'-TCC TGT GGA GAT ACC TTC ACC A-3' (sense) and 5'-CAG CTC AGT GAA GGT GAG AGA T-3' (antisense); rat tryptophan hydroxylase 1 (XM_341862) 5'-CAA GGA GAA CAA AGA CCA TTC-3' (sense) and 5'-ATT CAG CTG TTC TCG GTT GAT G-3' (antisense); rat high affinity IgE receptor, α -subunit (M21622) 5'-TGT GTA CTT GAA CGT GAT GCA A-3' (sense) and 5'-TGT CTA AGA CCA C GT CAG CAG-3' (antisense); *Fcer1* β -subunit (*Fcer1b*, M22923) 5'-TGT CCG AAA GGA AAA ACA CAC-3' (sense) and 5'-CGA TGC TGC TGA CAA TGT TT-3' (antisense); and *Fcer1* γ -subunit (*Fcer1g*; L04306), primer 5'-CCC TGC TCT ACT GTC GAC TCA-3' (sense) and primer 5'-TCT CAC GGC TGG CTA TGT CT-3' (antisense).

Western Blot Analysis—To detect Fc ϵ R1 α protein, tissues or cells were homogenized in 0.1 mM Tris-HCl (pH 7.5) containing protease inhibitor mixture (Roche Applied Science) using a tissue grinder. The homogenate was sonicated (three 1-s pulses, Bronwill Scientific, Rochester, NY), boiled (Laemmli sample buffer in a nonreducing condition), and clarified (centrifugation 6,000 \times g, 5 min). The proteins in the supernatant were separated by SDS-PAGE (12% precast gel, Invitrogen) and were then transferred onto an Immobilon-P membrane (Millipore, Bedford, MA). Fc ϵ R1 α was detected with monoclonal ER antibody (10 μ g/ml). The blots were stripped for subsequent detection with anti-actin antisera (1:1000 dilution, Sigma) to confirm equal loading (14). For Fc ϵ R1 γ protein detection, samples were run under nonreducing conditions in a 4–12% NuPage BisTris gel (Invitrogen), transferred to nitrocellulose membrane, and immunodetected with rabbit anti γ -subunit antisera (1:1000 dilution, Upstate Biotechnology).

RESULTS

***Fcer1a* mRNA and Fc ϵ R1 α Protein Occur in the Pineal Gland**—*Fcer1a* mRNA and Fc ϵ R1 α protein were detected in pineal glands removed at midnight (Fig. 1, A and B) but not in those removed at noon. A similar night/day difference was obvious from *in situ* hybridization (Fig. 2A). Northern blot and 5' rapid amplification of cDNA ends analysis (supplemental Fig. 1) indicated that the pineal transcript (~1.3 kb) was identical in size and sequence to that expressed in RBL-2H3 cells (GenBankTM accession number M21622) (11, 18). Western blot studies indicated that the immunopositive proteins in RBL cell and in the pineal gland were similar in size (~50kDa, Fig. 1B). The maximal level of expression of the *Fcer1a* mRNA occurs 5 h after the lights are turned off (midnight, ZT19, Fig. 1, C and D), and elevated expression persists during the remainder of the night period.

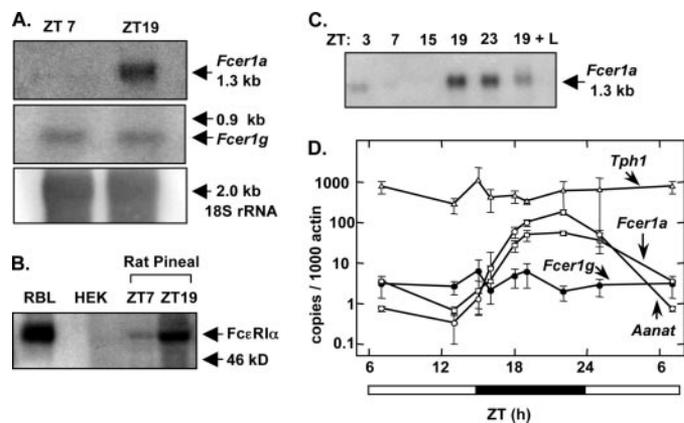


FIGURE 1. Expression of *Fcer1a* and *Fcer1g* mRNA and Fc ϵ R1 α protein in the rat pineal gland. A, expression of *Fcer1a* and *Fcer1g* genes in pineal gland collected at midnight and midday. Rats were maintained in a controlled lighting environment (light/dark 14:10). Tissues were collected from animals sacrificed at ZT7 (midday) and ZT19 (midnight). Total RNA extraction from tissues and subsequent Northern blot analysis were performed as described under "Experimental Procedures." Loading of similar amounts of total RNA (8 μ g) was verified by monitoring 18 S rRNA. B, daily rhythm of Fc ϵ R1 α protein. Fc ϵ R1 α protein was detected by Western blot analysis using monoclonal antibody (ER) as described under "Experimental Procedures." ~35 μ g of total pineal gland protein was loaded in each lane, and the immunodetection was performed with 10 μ g/ml ER antibody. Lysates prepared from RBL-2H3 (1×10^6 cells) and HEK293 (1×10^6 cells) were used, respectively, for positive and negative controls. C, daily change and light suppression of *Fcer1a* mRNA. Expression at ZT time is as indicated. For "ZT19 + L" samples, a set of animals at ZT19 were exposed to light for 30 min before being sacrificed. D, daily rhythms in *Fcer1a*, *Aanat*, *Tph1*, and *Fcer1g* mRNA. Quantitative reverse transcription-PCR was performed, and results are expressed as copies in a pineal gland per 1000 copies of actin at the indicated times. Results are mean \pm S.E. of three replicates.

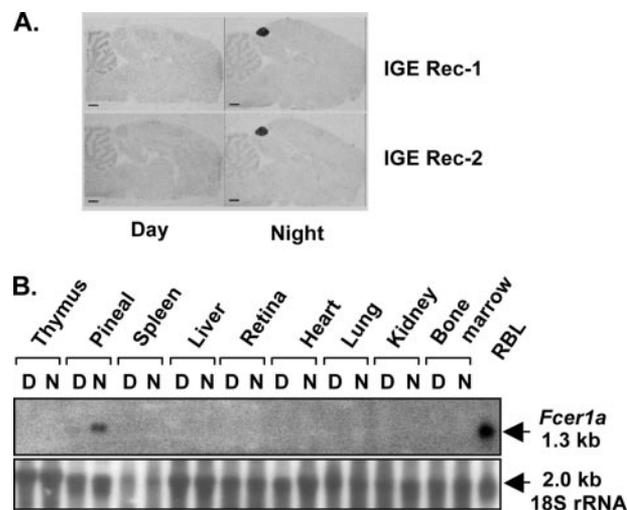


FIGURE 2. Distribution of *Fcer1a* mRNA. A, *in situ* hybridization of rat brain sections. Animals were maintained in a controlled lighting environment for 2 weeks with a 12:12 light/dark cycle. *In situ* hybridizations were performed using two probes, IgE rec-1 (upper panel) and IgE rec-2 (lower panel), as described under "Experimental Procedures." Left panels are brain sections prepared from animals sacrificed at midday (ZT6), and right panels are sections from animals sacrificed at midnight (ZT12). B, Northern blot analysis of selected peripheral tissues as indicated. Tissues were collected from rats sacrificed at midday and midnight. Four μ g of total RNA were loaded in each lane and were analyzed using a ³²P-labeled *Fcer1a* probe. For further details, see "Experimental Procedures."

For comparative purposes, the levels of transcripts of two well studied genes that are strongly expressed in the pineal gland are also presented, arylalkylamine *N*-acetyltransferase (*Aanat*) and tryptophan hydroxylase 1 (*Tph1*). The former is

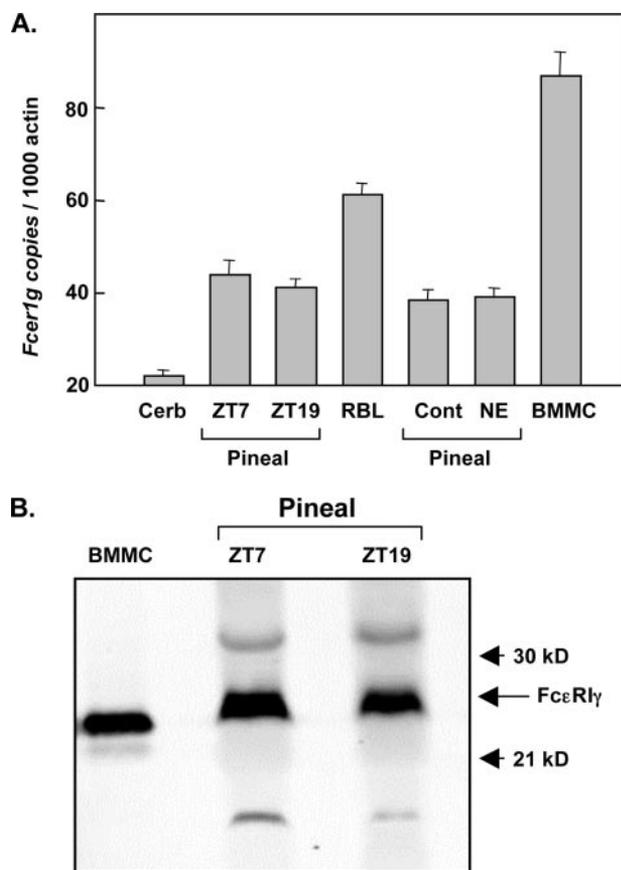


FIGURE 3. Expression of Fcer1g mRNA and protein in pineal gland. *A*, quantitative PCR analysis of Fcer1g mRNA expression. Pineal gland samples were prepared either from tissues extracted at ZT7 and ZT19 or from glands treated with or without NE in organ culture as described under "Experimental Procedures." cDNA samples from RBL-2H3 cells (RBL), bone-marrow mast cells (BMMC), and cerebellum (Cerb) were also included in the analysis. Cont, control. *B*, immunodetection of FcεRIγ protein in the pineal gland at ZT7 and ZT19. Samples (~100 μg) of pineal gland protein extract were loaded in each lane. Lysate prepared from mouse bone marrow-derived mast cells (3 × 10⁶ cells) were used as a positive control.

known to be highly rhythmic and the latter to be expressed at constant high levels (Fig. 1D). It was found that the 24-h patterns of *Aanat* and *Fcer1a* transcripts were quantitatively and qualitatively similar.

Pineal Fcer1a mRNA Levels Are Relatively High Compared with Other Tissues—The level of *Fcer1a* mRNA in the rat pineal gland was found to be greater than other tissues examined, as indicated by the results of *in situ* hybridization analysis (Fig. 2A) and Northern blot analysis (Fig. 2B). The latter included bone marrow, a source of mast cells in which the transcript was undetectable. Expression in the pineal gland was less intense than in the RBL-2H3 and in a culture of mouse bone marrow-derived mast cells. *Fcer1a* was also expressed in mouse pineal gland as demonstrated by end-point reverse transcription-PCR (supplemental Fig. 2), indicating that expression is not unique to the rat pineal gland.

The Pineal Gland Expresses FcεRIγ but Not FcεRIβ—The abundance of mRNA encoding the γ-chain of the FcεRI receptor was similar in glands removed during the day or night (Fig. 1, A and D), although expression was somewhat lower than in bone marrow-derived mast cells and RBL cells (Fig. 3A). FcεRIγ protein was also immunodetected at similar levels in the day

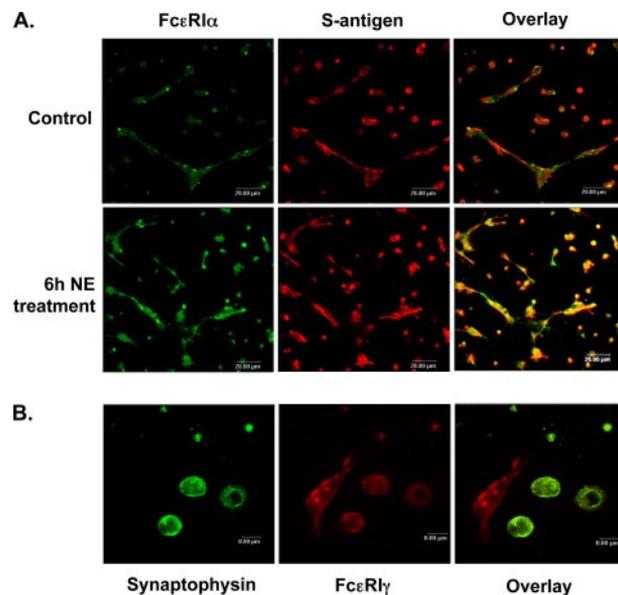


FIGURE 4. Immunocytochemistry using pinealocytes dissociated from rat pineal gland. Rat pinealocytes were dissociated from pineal glands and attached on slides (1 × 10⁶ cells/chamber) with overnight incubation at 37 °C as described under "Experimental Procedures." On the following day, cells were treated with 1 μM NE for 6 h. *A*, FcεRIα and S-antigen colocalization. Following treatment, cells were fixed, permeabilized, and dual-immunolabeled with anti-FcεRIα (green fluorescence, 10 μg/ml) and anti-S antigen polyclonal antibody (red fluorescence, 1:400 dilution) and detected by fluorescence microscopy as described under "Experimental Procedures." Control cells were cultured in the absence of NE. *B*, colocalization of FcεRIγ (red) and synaptophysin (green) and FcεRIγ in the pinealocytes. FcεRIγ and synaptophysin were detected using rabbit polyclonal anti-FcεRIγ (5 μg/ml) and monoclonal anti-synaptophysin (1:200 dilution). Yellow signals in the merged image (Overlay) represent the colocalization. For all dual label detections, a set of samples were treated only with second antibodies omitting the primary antibodies. No cross-reactivities were detected in any of the negative controls (data not shown). For further details see "Experimental Procedures."

and night pineal gland as a ~25 kDa band using nonreducing conditions (Fig. 3B). However, pineal *Fcer1b* mRNA was not detected using Northern hybridization and quantitative real-time PCR methods that detect the transcript in RBL cells (data not shown).

FcεRIα and FcεRIγ Are Expressed in the Pinealocyte—The rat pineal gland is composed primarily of pinealocytes (>90%), and it also includes parenchymal interstitial/glia cells, phagocytes, vascular pericytes, endothelial cells, and smooth muscle cells. Nonresident transiently present cells include leukocytes and immature mast cells (19, 20). To investigate whether FcεRIα and FcεRIγ proteins are expressed specifically in the pinealocyte, immunocytochemical analysis was done using dispersed cells. Pinealocytes were positively identified using antisera against either of two pinealocyte marker proteins, S-antigen or synaptophysin (21, 22).

FcεRIα protein was nearly undetectable in untreated cells. However, this was increased in nearly all cells following treatment with NE (1 μM, Fig. 4A), and most of the FcεRIα positive cells were also positive for S-antigen. FcεRIγ protein was detected in the synaptophysin-positive cells and in other cells (Fig. 4B). As with the immunoblot analysis, FcεRIβ protein was not detected in the pinealocytes. Moreover, there was no binding to the cells by a monoclonal antibody (mAb BC4), which detects the cell surface expression of FcεRI (data not shown).

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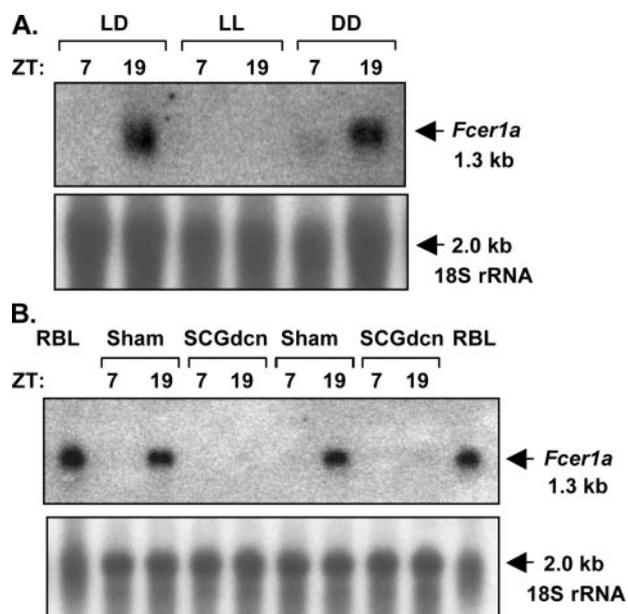


FIGURE 5. Daily rhythm of Fc ϵ R1 α mRNA expression is circadian and is regulated by neural input into pineal gland. *A*, Fc ϵ R1 α mRNA rhythm is circadian in nature. Northern blot analysis was performed using pineal gland total RNA (4 μ g) prepared from animals sacrificed at ZT7 and ZT19 that were either kept under normal light/dark (14:10, LD) cycle, constant darkness (DD), or constant light (LL) conditions for 3 days. *B*, disrupting the SCN to pineal circuit by bilateral decentralization of the SCG abolishes the induction of the Fc ϵ R1 α gene expression in the pineal gland. Total RNA from glands obtained at ZT7 and ZT19 were collected from sham-treated (Sham) and bilateral SCG decentralized (SCGdcn) animals, and Fc ϵ R1 α mRNA was detected by Northern blot. As a positive control, total RNA preparation from RBL-2H3 cells was used. Each lane was loaded with 4 μ g of total RNA. A 18S rRNA probe was used to verify equal loading.

These results demonstrate that Fc ϵ RI α and Fc ϵ RI γ proteins occur in the pinealocyte albeit not on the cell surface.

Regulation of Fc ϵ R1 α mRNA and Protein: Circadian and Photoneural Control—Circadian changes in pineal function are driven by the central circadian oscillator in the suprachiasmatic nucleus (SCN). It is hardwired to the pineal gland and generates pineal rhythms in animals in the absence of lighting cues in constant darkness. Here we found that the daily rhythm in Fc ϵ R1 α mRNA persists in animals housed in constant darkness for 3 days (DD, Fig. 5A), indicating that the day/night rhythm in Fc ϵ R1 α mRNA is circadian in nature and does not require light-dark transitions.

The transmission of signals from the SCN to the pineal gland is gated by light acting downstream of the SCN. To determine whether the Fc ϵ R1 α mRNA levels were under photic control, animals were kept in constant light for 3 days. This blocked the nocturnal increase in Fc ϵ R1 α mRNA (Fig. 5A, LL). In addition, a 30-min exposure to light at night (ZT19) rapidly reduced the abundance of Fc ϵ R1 α mRNA (Fig. 1C and supplemental Fig. 3). Neural signals from the SCN to the pineal gland pass through the superior cervical ganglia (SCG), from which postganglionic processes send projections to the pineal gland (9, 19, 23, 24). To determine whether neural stimulation via the SCG is required for rhythmic expression of Fc ϵ R1 α in the pineal gland, the SCG was surgically decentralized. This blocked the nocturnal increase in pineal Fc ϵ R1 α mRNA (Fig. 5B). Accordingly, these findings support the interpretation that the rhythm in expression of this gene is regulated by the same neural system that

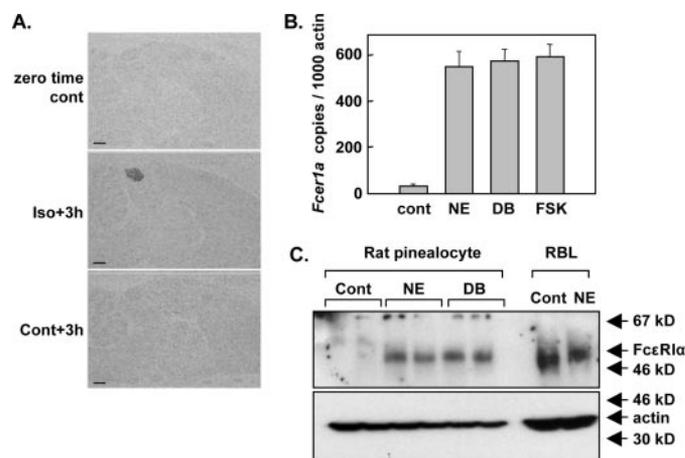


FIGURE 6. Fc ϵ R1 α mRNA and protein are regulated by adrenergic/cAMP stimulation. *A*, ISO induces Fc ϵ R1 α mRNA in rat pineal glands *in vivo*. Rats were injected intraperitoneally with β -adrenergic agonist ISO (5 mg/kg rat) at ZT6 and were sacrificed at ZT9 (ISO + 3h, 12:12 light/dark). As controls, rats were either sacrificed at ZT6 (zero time cont) or were injected with saline at ZT6 and sacrificed at ZT9 (Cont+3h). Median sections of the rat brains were subjected to *in situ* hybridization as described under "Experimental Procedures" using 35 S-labeled Fc ϵ R1 α probe (mixed IgE-rec1 and IgE-rec2 probes). *B*, real-time quantitative PCR analysis of Fc ϵ R1 α mRNA in pineal glands from organ culture. Glands were treated for 6 h with NE (1 μ M), dibutyryl cyclic AMP (DB, 1 mM) or forskolin (FSK, 10 μ M). Results are calculated from mean \pm S.E. of 4 replicates. *C*, adrenergic agonist and cAMP antagonist regulate Fc ϵ RI α protein in cells. Pinealocytes (2×10^6 cells in 250 μ l Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum) dissociated from day-sacrificed rat pineal glands and grown in suspension and RBL-2H3 cells (2×10^6 cells in 250 μ l Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum) were treated with 1 μ M NE and 1 mM dibutyryl cyclic AMP (DB) for 6 h separately. Untreated cells that had been incubated for 6 h were used as controls. Following treatment, cells were harvested, lysed in 2 \times SDS-sample buffer in nonreducing condition, sonicated briefly (2 \times 2-s pulses, Bronwill Scientific, Rochester, NY), and boiled for 5 min. Samples were run on a 10% SDS-PAGE and immunoblotted with anti-Fc ϵ RI α and anti-actin antibodies. For further details see "Experimental Procedures."

regulates melatonin production and many other elements of mammalian pineal function (14, 25–27).

Adrenergic/cAMP Control of Fc ϵ RI α mRNA and Protein—Projections from the SCG stimulate the pineal gland by releasing norepinephrine at night (23). This in turn activates the adrenergic signaling cascade in the pineal gland by activating adenylate cyclase via a mechanism involving β -adrenergic receptors (26). To investigate whether β -adrenergic stimulation induces the Fc ϵ R1 α mRNA, rats were injected with the β -adrenergic agonist ISO at ZT6 (midday). This increased Fc ϵ R1 α mRNA in the pineal gland within 3 h (ZT9, Fig. 6A).

It was also found that *in vitro* adrenergic stimulation with NE elevated Fc ϵ R1 α expression (Fig. 6B). To investigate whether the adrenergic stimulation of Fc ϵ R1 α expression might involve cAMP, pineal glands were treated (6 h) with either the cAMP antagonist dibutyryl cyclic AMP (1 mM) or the adenylate cyclase activator forskolin (1 μ M). Both treatments increased Fc ϵ R1 α mRNA (Fig. 6B). NE or dibutyryl cyclic AMP treatment of isolated pinealocytes also increased Fc ϵ RI α protein as detected by Western blot (Fig. 6C). Together with the known stimulatory effects of NE on pinealocyte cAMP, these observations are consistent with the conclusion that that Fc ϵ R1 α expression in the pinealocyte is regulated by an adrenergic/cyclic AMP mechanism.

DISCUSSION

The discovery of high expression of both Fc ϵ RI α and Fc ϵ RI γ in the pineal gland provides tantalizing evidence that Fc ϵ RI may have a physiological role in the pineal gland. Further support for the view that Fc ϵ RI could function in the pineal gland comes from the finding that several molecules associated with Fc ϵ RI signaling in the mast cell are also expressed in the pineal gland, including Lyn kinase and Fyn kinase.⁴ In addition, serotonin, which is released by IgE-mediated mast cell activation, is robustly synthesized in the pineal gland resulting in very high concentrations (~ 0.5 mM) (28).

The absence of Fc ϵ RI β in the pineal gland is of interest in view of the evidence that a plasma membrane-associated tetrameric receptor cannot form without this subunit (7). This raises the possibility that the expression of this subunit is under independent control and that the functional tetrameric receptor formation is regulated by Fc ϵ RI β availability. For example, it might be regulated by a cytokine that is induced by immune stress. This hypothesis was tested either by treating pinealocytes with a mixture of cytokines or by initiating experimental pinealitis and uveitis (supplemental data). However, neither treatment induced expression of the β -chain. It is also possible that a functional trimeric ($\alpha\gamma_2$) intracellular Fc ϵ RI complex forms as appears to be the case in eosinophils, monocytes, and antigen-presenting cells (6, 8, 29). These cells participate in IgE-dependent processes. Accordingly, a functional response could be generated if an IgE and allergen were translocated to the cytoplasm by the low affinity IgE receptor as occurs in the intestine (30). The pineal gland is not protected by the blood-brain barrier (31) making it possible for IgE and other molecules to enter pineal perivascular space. The delivery of antigens to pinealocytes might be facilitated by resident antigen-presenting phagocytes (32). Moreover, the possibility that IgE can be translocated into the pinealocyte is supported by evidence that the low affinity IgE receptor (CD23) is expressed in the pineal gland⁴ and that *PepTI*, which encodes a peptide transporter, is expressed in the pineal plasma membrane at night (27). Based on these observations, it is not unreasonable to consider that allergens/IgE could enter the pinealocyte, triggering pineal responses through binding to soluble high affinity IgE receptor in the cytoplasm.

A striking feature of the expression of pineal *Fcer1a* expression is the large night/day difference that has not been reported previously in any cell. The large changes in transcript number appear to be driven by the neural system that controls melatonin production in the pineal gland (9). Whereas this is a pineal gland-specific control mechanism, cyclic AMP, the second messenger mediating control of *Fcer1a*, is ubiquitous. This is significant because cyclic AMP might impact *Fcer1a* expression in other cells.

The dynamic nature of the changes in *Fcer1a* expression not only reflects changes in cyclic AMP but is also dependent on undefined mechanisms that rapidly destroy the transcript after cyclic AMP-dependent ceases. This rhythmic pattern of tran-

script abundance appears to be translated immediately into a rhythm in the encoded protein. Moreover, the relatively rapid disappearance of the protein may reflect an inherent unstable nature of Fc ϵ RI α protein in the cytosol and to rapid proteolysis, perhaps involving the proteasome as is the case with pineal arylalkylamine *N*-acetyltransferase (33). The stable nature of Fc ϵ RI α protein in the membrane in other cells appears to reflect both association with other subunits and glycosylation (34–36); apparently, one or both events do not occur in the pinealocyte under the conditions examined in this study.

A daily rhythm in the production of Fc ϵ RI α protein would provide the opportunity to generate new IgE·Fc ϵ RI complexes every day, thereby providing an “up-to-date” reflection of circulating allergens and IgEs. The advantage of such a short memory immune response is not clear; however, it could mediate generation of a signal indicating that there has been a change in immune status because IgE·Fc ϵ RI complexes on other cells are long lived.

In addition to being potentially linked to the resident phagocytes, a pineal IgE·Fc ϵ RI system might initiate T-cell-mediated inflammatory response. It is known that T-cells are recruited into the chicken, mouse, and monkey pineal gland (37, 38). Although the mechanism underlying this is unknown, it might reflect penetration through the vasculature and be influenced by the release of serotonin and cytokines from pinealocytes. T-cells in the pineal extracellular space would be exposed to high concentrations of pineal products, including serotonin, melatonin, and other serotonin derivatives. The effects of most of the serotonin derivatives on lymphocytes are not known. However, melatonin has been proposed to act as an immune modulator that enhances lymphocyte proliferation and cytokine production (39–43). The effects of exposure to high concentrations of pineal products are difficult to predict and deserve investigation.

Another potential set of signaling molecules in the pineal perivascular space are dopamine and NE, the transmitters of the sympathetic nervous system that are released at night. The latter is of some special interest because IgE production in B-lymphocytes is stimulated by NE and the mechanism involves a cAMP-p38MAPK pathway (44, 45). Accordingly, B-lymphocytes invading the pineal gland might be induced to secrete IgE that would then be available at high local concentrations for interactions with pinealocytes. The effects of dopamine are not known.

A third set of molecules that could act on recruited lymphocytes may reside in pinealocyte dense core vesicles (20). These have not been fully characterized but appear to contain glutamate (20). The contents of these vesicles could be released via activation of the Fc ϵ RI complex.

The discovery that pinealocytes express components of the high affinity IgE receptor regulated by circadian adrenergic/cAMP mechanism is of special value because it provides investigators with a molecular foundation to obtain a better understanding of the role of the pineal gland in the immune response, which could lead to novel drug targeting strategies in allergy treatments.

⁴ S. Ganguly and D. C. Klein, unpublished observations.

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