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Development of MEKA (phosducin), Gβ, Gγ and S-antigen in the rat pineal gland and retina

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Pinealocytes and retinal photoreceptor cells contain an unusual cytoplasmic complex composed of the Gβγ dimer of GTP-binding regulatory proteins (G-proteins) tightly bound to an acidic 33 kDa phosphoprotein termed MEKA or phosducin; MEKA is a substrate of cyclic AMP-dependent protein kinase. This study characterized the developmental appearance of these and two related proteins, Gγ and S-antigen, in pineal and retinal tissue. MEKA was absent in the pineal gland prior to birth, at a time when it was possible to detect Gβ in pineal cytoplasm, indicating that the appearance of Gβ in the cytoplasm precedes that of MEKA and does not appear to require the presence of MEKA. The absence of MEKA at this time indicates that the cyclic AMP stimulation of pineal serotonin *N*-acetyltransferase activity is not mediated by MEKA, which has been considered as a possible role of MEKA. After postnatal day 7, pineal MEKA and cytoplasmic Gβ increased in a parallel manner, with peak values occurring at about postnatal day 21. Thereafter, both proteins in the pineal gland decreased in a parallel fashion to 10 and 35% of their peak values, respectively; in contrast, the cytoplasmic protein S-antigen and membrane associated Gβ remained at maximal levels after this time. Whereas both MEKA and Gβ decreased late in development in the pineal gland, these proteins either increased or remained constant in the retina. These tissue-specific patterns were found to differ from those of another cytosolic protein found exclusively in the pineal gland and retina, S-antigen, which remained constant after day 21 in the pineal gland but decreased in the retina late in life.

INTRODUCTION

The mammalian pineal gland and retina are thought to have evolved from a common ancestral photochemical transduction system which converted darkness into a chemical signal-melatonin (for a review see ref. 23). This view is founded on anatomical evidence[24] and on growing biochemical findings which indicate that these tissues express a set of proteins not found elsewhere. These proteins include some commonly associated with transmembrane signal transduction, specifically opsin[3,12], opsin kinase[32] and interretinal retinoid binding protein[6]. Others are associated with intracellular signal transduction including S-antigen (arrestin)[11] or are involved in melatonin synthesis, including arylalkylamine *N*-acetyltransferase[22] and hydroxyindole-O-methyltransferase[34].

The most recent addition to the growing list of pineal/retinal proteins is MEKA, an acidic 33 kDa substrate of cyclic AMP-dependent protein kinase, also termed phosducin and 33 K protein. This phosphoprotein is especially interesting because it exists tightly bound in the cytoplasm to the β subunit of GTP-binding regulatory proteins (G-proteins), yet shares no homology with the α subunit of the G-protein which stimulates adenylylcyclase (Gsα) or other G-proteins[1,13,17,26]. Studies on the retina indicate this complex also contains the γ subunit of the GTP-binding regulatory protein transducin (Gtγ)[16]. The functional importance of the MEKA/Gβ complex has not been

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clearly established, nor has the effect of phosphorylation. However, it seems reasonable to suspect that it is involved in cyclic AMP signal transduction, perhaps the adrenergic → cyclic AMP stimulation of arylalkyamine *N*-acetyltransferase activity.

In the experiments presented here the development of the MEKA, Gβ and Gγ was characterized in the rat pineal gland and retina for several reasons. First, it was of interest to determine whether Gβ might be present in the cytoplasm at times when MEKA and Gγ are absent, or if these proteins exhibited identical developmental patterns. Second, the function of MEKA in the pineal gland is unknown. The appearance of this protein might be associated with the developmental appearance of a tissue specific function. For example, if MEKA plays a critical role in cyclic AMP stimulation of arylalkylamine *N*-acetyltransferase activity, it should be present at all times during development when cyclic AMP stimulation of the enzyme can be demonstrated. The developmental appearance of S-antigen was also studied to provide an additional measure of the developmental appearance of pineal/retinal proteins.

MATERIALS AND METHODS

Animals

The animals used were Sprague–Dawley rats (Zivic-Miller Lab, Allison Park, PA, USA). They were housed under a lighting cycle providing 14 h light and 10 h darkness with the light period starting at 07.00 h (LD 14:10). Food and water were provided ad libitum. Rats of known age or conception date were decapitated as required between 10.00 and 15.00 h, and their pineal gland and/or retina were rapidly removed and placed on solid CO_2. Male rats were used for ages 14 days and older; mixed sexes were used at younger ages. For each graph, tissue was collected from two to three experiments.

Preparation of pineal cytosol and membranes

Cytosolic preparations were routinely prepared by sonication of tissue (preliminary results did not show any difference between results obtained by either homogenization or sonication) in 10 volumes (v/w) of buffer. Pineal gland weight at different ages was based on published data[9]; frozen retinal tissue was weighed rapidly. Each pineal preparation was a pool of tissue from 10 to 40 animals obtained from a single collection. Retinal preparations were pools of tissue from 4 to 8 animals. In each collection there were at least two tissue pools for each time point. The following buffer (buffer A) was used: 0.5 mM EDTA, 0.5 mM EGTA, 0.5 mM phenylmethylsulfonyl fluoride, 21 μM leupeptin, 20 mM Tris-HCl, pH 7.4. The homogenate was then centrifuged (4°C, 1 h, 100,000 × *g*). The supernatant was removed and used subsequently as cytosol. The tube was washed once with buffer A and the pellet was then resuspended in a volume of buffer A equal to the starting volume; this preparation was subsequently used for analysis of membrane proteins.

Polyacrylamide gel electrophoresis of cytosol and membrane preparations

Protein in cytosolic and membrane preparations was measured by a dye-binding method[5]. Samples contained an amount of protein that was equivalent to the minimum amount of protein in adult tissue required for immunodetection by the antibody. Samples (30 μl) were prepared by adding an equal volume of sample buffer (0.125 mM Tris-HCl, pH 6.8, 4% sodium dodecyl sulfate (SDS), 20% glycerol, 0.016% Bromophenol blue, 0.002% pyronine, and 10% 2-mercaptoethanol) and heating (100°C, 5 min). SDS-Slab gel electrophoresis was performed using 12.5% acrylamide/bis-acrylamide (30:1) according to the method of Laemmli[15]. The running buffer was 25 mM Tris, pH 8.0, 190 mM glycine, and 0.1% SDS. Samples were run using a vertical gel electrophoresis apparatus (Bethesda Research Laboratories, Gaithersburg, MD) at 80 V for 2 h and at 180 V for 3 h. The above procedure was used routinely, except when Gγ was studied, in which case tricine buffer and a 10% gel were used[31].

Electroblotting

Proteins were transferred from gels onto PVDF membranes (Immobilon P, Millipore Corp., Bedford, MA)[35]. A commercial blotting assembly (BioRad Trans-blot cell) was used with the following buffer: 25 mM Tris, 190 mM glycine, pH 8.3, and 20% methanol. Apparent molecular weights were estimated using prestained standards (Rainbow Standards, Amersham): myosin (200 kDa), phosphorylase b (92.5 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (21.5 kDa) and lysozyme (14.3 kDa). Electrophoretic transfer was accomplished at 150 mAmp for 8 h at room temperature.

Immunodetection

Electroblots were blocked with 0.3% Tween-20 in phosphate buffered saline (3 mM Na_2HPO_4, 0.9 mM KH_2PO_4, 154 mM NaCl, pH 7.5) for 30 min and incubated with antiserum in Tris-buffered saline (20 mM Tris HCl, 500 mM NaCl, pH 7.5) containing 10% goat serum for 14 h at room temperature (antisera dilutions appear in figure legends). The blots were then washed 3 times with 0.05% Tween in Tris buffered saline (TTBS), and incubated with goat anti-rabbit antiserum (1/1600) conjugated to alkaline phosphatase. The immunoreaction was visualized by two methods. Autoradiographic detection was used for the quantitation presented in the figures. Blots were exposed to ^{125}I-protein A (100,000 cpm/ml TTBS) for 1 h, washed with TTBS (5 changes, 1 h) and dried. Autoradiography was performed with Kodak X-Omat AR2 film at −70°C with an intensifying screen (Cronex Lighting Plus DuPont). A chromogenic method was also used in which blots were incubated with 0.2 mg/ml Nitro blue tetrazolium, 0.2 mg/ml 5-bromo 4-chloro-3-indolyl phosphate in 5 mM $MgCl_2$, 10 μM $ZnCl_2$, 1% methyl sulfoxide and 100 mM Tris, pH 9.7. The results of both methods were in general agreement. However, the results of the autoradiographic analysis are presented because this method was more sensitive and allowed quantitation of the intensity of a broader range of immunoreactions.

Quantitation of immunoreactive proteins on blots

Autoradiograms were visualized by a CCD camera (Sierra Scientific) above a light box of variable intensity (Illuminator model 890, Imaging Research Inc). The density of immunopositive bands was measured with the IMAGE program running on a Macintosh II[25]. Typically at least four pools of pineal tissue and two pools of retina were analyzed; in the case of the one year pineal gland data, only two groups were used. In certain cases, the range of immunoreaction was too broad to allow precise quantitation of all ages studied. In these cases two exposures were normalized to each other; normalization was based on the optical density (O.D.) of several bands of subsaturating intensity on both autoradiograms. Images generated by the alkaline phosphatase reaction were analyzed with a BioRad model 620 densitometer and the 1-D Analyst software package (BioRad, Rockville Center, NY). In addition, the data from each analysis were normalized so that the maximum O.D. was equal to 100%. Pooled data is presented in the figures; pineal data are based on tissue from at least four collections and retinal data on tissue from two. Data are presented as the mean O.D. ± S.E.

To compare the relative amounts of immunoreactive proteins in two tissue preparations (see Table I) a range of amounts of each preparation was loaded on the same gel and analyzed. For this purpose homogenates prepared from tissues removed from 60-day-old rats were used.

Materials

Acrylamide, bisacrylamide, SDS, TEMED, ammonium persulfate and other chemicals for electrophoresis were purchased from Bio-Rad. Phenylmethylsulfonyl fluoride, leupeptin, EDTA, EGTA, 5-bromo-4-chloro 3-indolyl phosphate and Nitro blue tetrazolium were purchased from Sigma (St Louis, MO). The following antisera were used: rabbit anti-$G\beta_{330-340}$ serum (SW/J75; prepared according to the method used to generate the RM antisera described by Simonds et al.[30]; Simonds, unpublished results), rabbit anti-Gγ (SE)[31], rabbit anti-Gγ serum (PE)[31], rabbit anti-bovine $MEKA_{65-96}$ serum (Anti-32)[1,28], and rabbit anti-bovine S-antigen serum (NEI04111083; Igal Gery, National Eye Institute).

RESULTS

Developmental profile of MEKA

The development of MEKA was exclusively studied in the cytosol, which contains more than 90% of total MEKA in adult pineal and retina (Table I).

MEKA was first detectable in the pineal and retina about one week after birth and reached a maximal level at day 21 (Fig. 1). Pineal MEKA levels decreased dramatically after day 21; day 60 and 360 O.D. values were 10 to 15% of the day 21 value. In contrast, the level of retinal MEKA did not change significantly between day 21 and day 60. The development of MEKA in rat retina is similar to that of MEKA in mouse retina[14,18], whereas the pineal MEKA developmental profile is similar to that of MEKA in mice with retinal degeneration[14,18].

In addition to the strong 33 kDa signal, the retina was found to contain two immunopositive bands of lower molecular weight (24 kDa and 27 kDa); these are prominent in fetal and neonatal tissue and disappear later in development. These two bands may reflect non-specific cross-reactivity of Anti-32 antiserum with unrelated retinal proteins, degradation products or potentially important proteins containing epitopes shared with MEKA.

Comparison of the relative amounts of MEKA in the adult rat pineal cytosol and retinal cytosol indicated that the retina contains approximately twice that in the pineal cytosol. (Table I).

Developmental profile of Gβ (Fig 2)

In contrast to pineal MEKA, which was not detectable prior to birth, Gβ was detected in pineal cytosol on embryonic day 19. Starting on day 5, cytosolic Gβ immunoreactivity increased markedly in parallel with the increase in MEKA. There was a 10-fold increase in the O.D. of the Gβ immunoreaction between day 0 and day 21. As was seen with MEKA, cytosolic Gβ decreased after day 21 to 35% of the day 21 level. At one year of age there were obvious differences between the developmental pattern of retinal and pineal cytosolic Gβ. Retinal Gβ did not decrease following day 21 and it increased between day 60 and 360. These developmental profiles of cytosolic Gβ in the rat retina and pineal are somewhat similar to those in the normal mouse retina and in the degenerative mouse retina, respectively[18].

The developmental appearance of cytosolic Gβ immunoreactivity (Fig. 2A) was compared to that of membrane Gβ (Fig. 2B). The developmental profile of pineal membrane Gβ is characterized by relatively constant levels, with near maximal levels occurring at the earliest time point.

It is interesting to mention that the adult rat retina membrane contains about 20-fold more Gβ compared to adult pineal membranes (Table I). The retina membrane and cytosol fractions contain similar amounts of Gβ. However, adult rat pineal membrane fraction contains 3-fold the amount of Gβ present in adult rat pineal cytosol (Table I). If the species of Gβ present in the cytosol and membrane represent the same molecu-

TABLE I

Relative distribution of MEKA, Gβ, Gγ and S-antigen in the adult rat pineal gland and retina

Samples of retina and pineal membrane (M) and cytosol (C) containing 1 to 100 μg of total protein were analyzed for MEKA, Gβ, Gγ and S-antigen immunoreactivity, as described in Materials and Methods. The values are based on 48 h film exposure for MEKA, Gβ and Gγ and 18 h film exposure for S-antigen. The relative quantities were estimated as described in Materials and Methods and the legends to Figs. 1–4. The O.D. ratio values can be interpreted to indicate the relative amount of sample required to produce the same O.D. i.e. a sample of pineal membrane containing 100 μg of total protein is required to generate the same MEKA signal produced by a sample of pineal cytosol containing 5 μg of total pineal. N.D., not determined because amount of protein in one or both samples was nearly undetectable.

	Ratio of total O.D.		*Ratio of specific concentrations (O.D. units / μg protein)*	
	$Retina_C$: $Retina_M$	$Pineal_C$: $Pineal_M$	$Retina_C$: $Pineal_C$	$Retina_M$: $Pineal_M$
MEKA	> 50:1 [1]	> 50:1	2:1	N.D.
Gγ	1:1	N.D. [2]	N.D. [2]	N.D. [2]
Gβ	1:1	1:3	60:1	20:1
S-Antigen	2:1	> 10:1	4:1	> 20:1

[1] The retinal membrane MEKA signal was very weak and the Western blot contained a number of non-specific bands of similar intensity.
[2] Pineal material produced a non-specific pattern after extended development for Gγ detection.

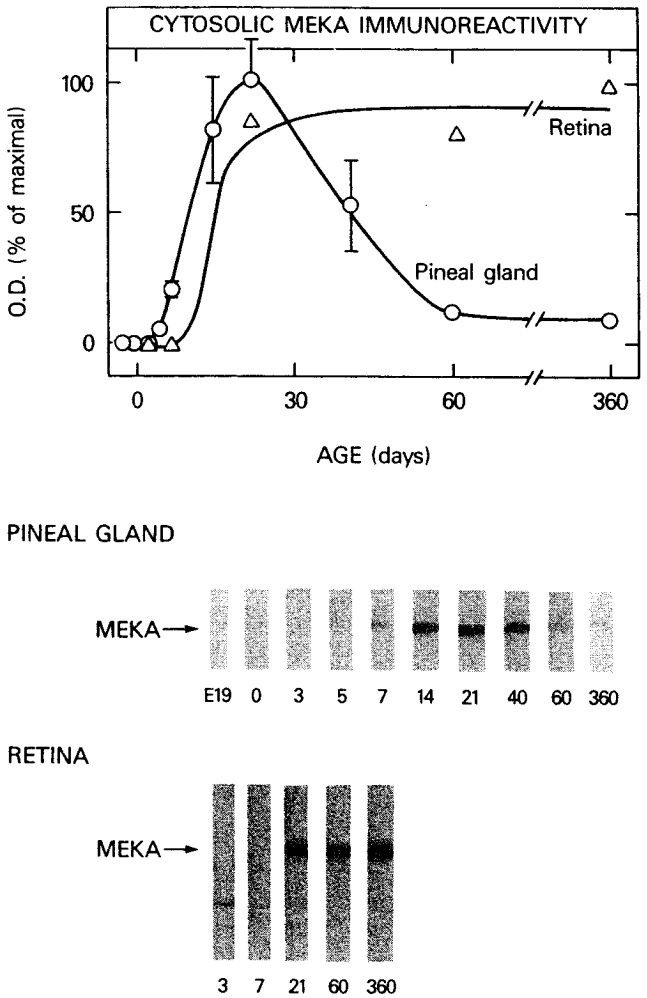


Fig. 1. Developmental profile of MEKA. Pineal (10 μg) and retina (10 μg) cytosolic preparations from rats at the indicated ages were analyzed. MEKA was detected using a rabbit anti-bovine $MEKA_{65-96}$ serum (Anti-32[1,28], 1:5,000). Normalized data are presented above; the 100% value is the highest value for each tissue. Photographs of typical immunoreactive bands are presented below. Quantitation was performed as described in Materials and Methods. The pineal data are based on material prepared from four tissue pools obtained in three experiments; each time point represents the mean value of % of maximum O.D. $\pm$ S.E. The absence of a S.E. bar indicates the error fell within the area of the symbol. The retinal data are based on material prepared from two collections of tissue; data are presented as the average of the % of maximal value in each experiment, which were within 20% of the mean. For further details see Materials and Methods.

lar species, then it would appear that the relative paucity of Gβ in pineal cytosol early in development is not due to low levels of synthesis. However, this might be the case in the retina, where both membrane Gβ and cytosolic Gβ are undetectable at birth and become detectable at day 21.

Developmental profile of GTγ and brain Gγ (Fig. 3)

It was not possible to detect GTγ in pineal cytoplasm or membranes with confidence. However, retinal cytosolic and membrane associated GTγ gradually increased from day 7 to 1 year of age (Fig. 3) *. Retinal GTγ was evenly distributed between the membrane and cytosol (Table I).

The absence of GTγ in the pineal raised the question of whether the pineal gland contains the Gγ present in brain. This was investigated and it was found that the Gγ common in brain was undetectable in the pineal gland at any time point **. Since Gβ is normally found tightly bound to Gγ which is essential for the membrane attachment of $\beta\gamma$ dimer[31]. We suspect that the pineal gland may contain a form of Gγ which is distinct from GTγ and brain Gγ.

Developmental profile of S-antigen (Fig. 4)

S-Antigen is a soluble retinal/pineal protein and it was of interest in this series of studies to monitor the levels of this protein for comparative purposes. Pineal S-antigen first appeared at birth, reaching a maximal level on day 21, in general agreement with histochemical data in the literature[8]. In contrast to the marked decrease in MEKA observed after day 21, S-antigen remained at elevated values. This result is in general agreement with data in the literature[8].

Retinal cytosolic S-antigen was first detectable at day 7. O.D. values increased 4-fold between day 7 and day 60. This result is in agreement with previous reports[8,18]. Following day 60 there was gradual decrease in immunoreactivity, differing from the pattern in the pineal. The O.D. values of S-antigen in the adult retina were about 50% of those from day 60 samples. The decrease in retinal S-antigen levels may be age related or may reflect a selective loss of the protein due to long term exposure to artificial light.

The adult rat retina cytosol contains 4-fold more S-antigen compared to the adult rat pineal cytosol (Table I). In the adult rat retina 67% of the S-antigen is present in the cytosol and 33% in the membrane while in the adult rat pineal gland more than 90% of the total S-antigen is located in the cytosol and less than 10% in the membrane (Table I).

* The immunoblot pattern of retinal GTγ was clear and unambiguous with a single strong band present at the predicted size. Pineal material produced a non-specific pattern after extended development.

** Unpublished data using a rabbit anti brain Gγ serum (PE)[31].

DISCUSSION

One of the questions which stimulated this investigation was whether or not the developmental appearance of cytosolic MEKA and $G\beta\gamma$ complex is coincident. A second issue which stimulated this investigation was whether MEKA was expressed at a high level early in development, when the system required for cyclic AMP stimulation of arylalkylamine *N*-acetyltransferase activity is functional. These issues will be addressed sequentially; in addition, the development of other proteins studied in this investigation will be discussed briefly.

$G\beta$ is typically found exclusively associated with membranes, and it seems reasonable to suspect that the atypical presence of $G\beta$ in pineal and retinal cytoplasm may be due to the presence of MEKA. Our results indicate that $G\beta$ is present in the cytosol of the pineal gland early in development when MEKA was not present. This is in agreement with published retinal data[18]. Four distinct β-subunit isotypes have been found[29]. Therefore, it is possible that the $G\beta$ present in the prenatal pineal may or may not be the same form that is present in the adult pineal and retina and that the membrane and cytosolic $G\beta$ represent different subtypes.

Assuming that the immunoreactivity of a protein does not change during development, our results indicate there are marked developmental changes in the relative amounts of the protein studied. The adult

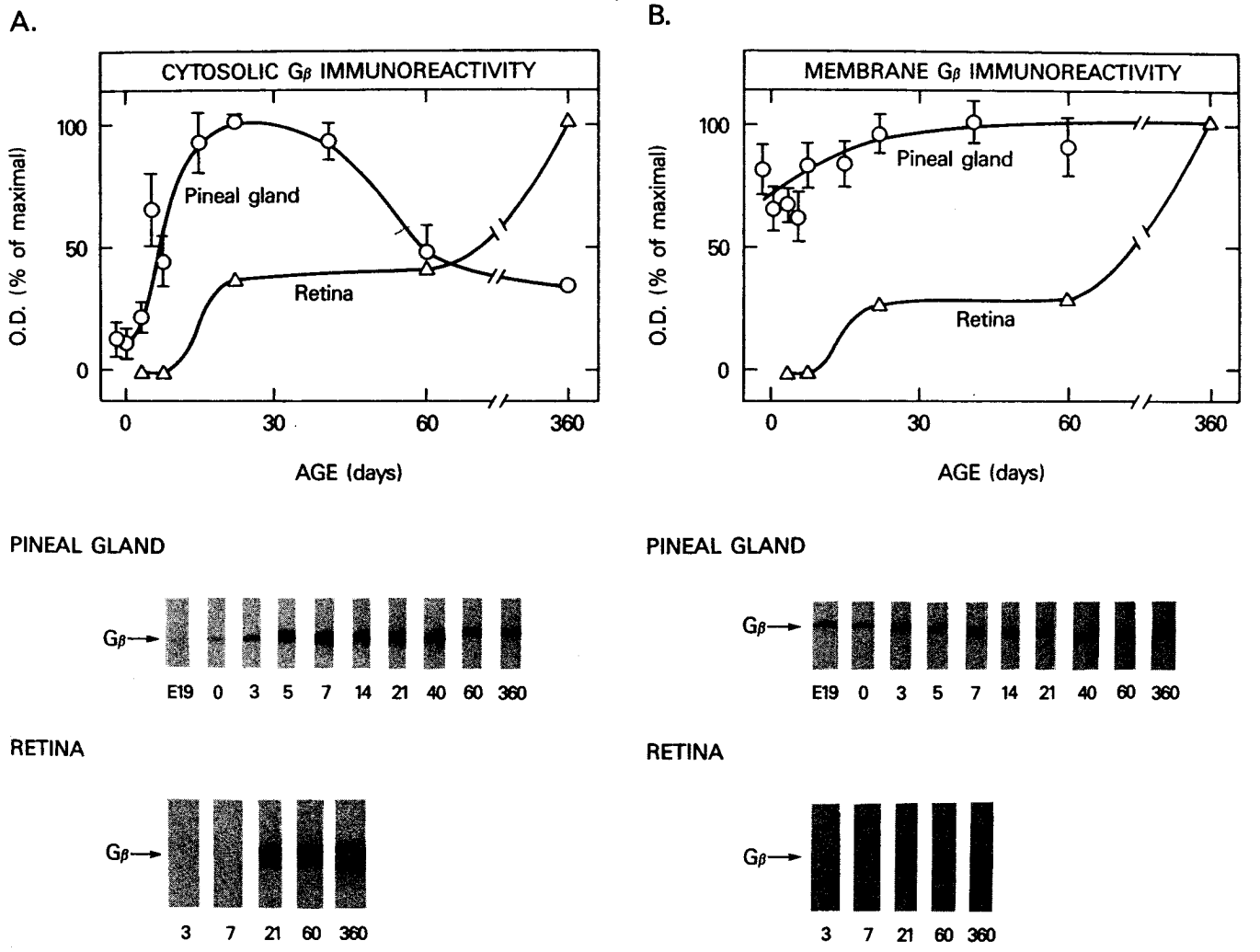


Fig. 2. Developmental profile of cytosolic and membrane $G\beta$. Pineal (30 μg) and retina (10 μg) cytosolic preparations (left panel) or membrane preparations 50 μg and 10 μg, respectively (right panel) from rats at various developmental stages were analyzed. $G\beta$ was detected using a rabbit anti-$G\beta_{330-340}$ serum (SW/J75; Simonds, personal communication; 1:500). Normalized data are presented above; the 100% value is the highest value for each tissue. Photographs of typical immunoreactive bands are presented below. Quantitation was performed as described in Materials and Methods. The pineal data are based on material prepared from five tissue pools obtained in three experiments; each time point represents the mean of the results of the analysis of each pool. Data are presented as the mean of % of maximum O.D. $\pm$ S.E. The absence of a S.E. bar indicates the error fell within the area of the symbol. The retinal data are based on two pools of retinae obtained from one experiment; data are presented as the average of the % of maximal value in each experiment (values were within 20%). For further details see Materials and Methods.

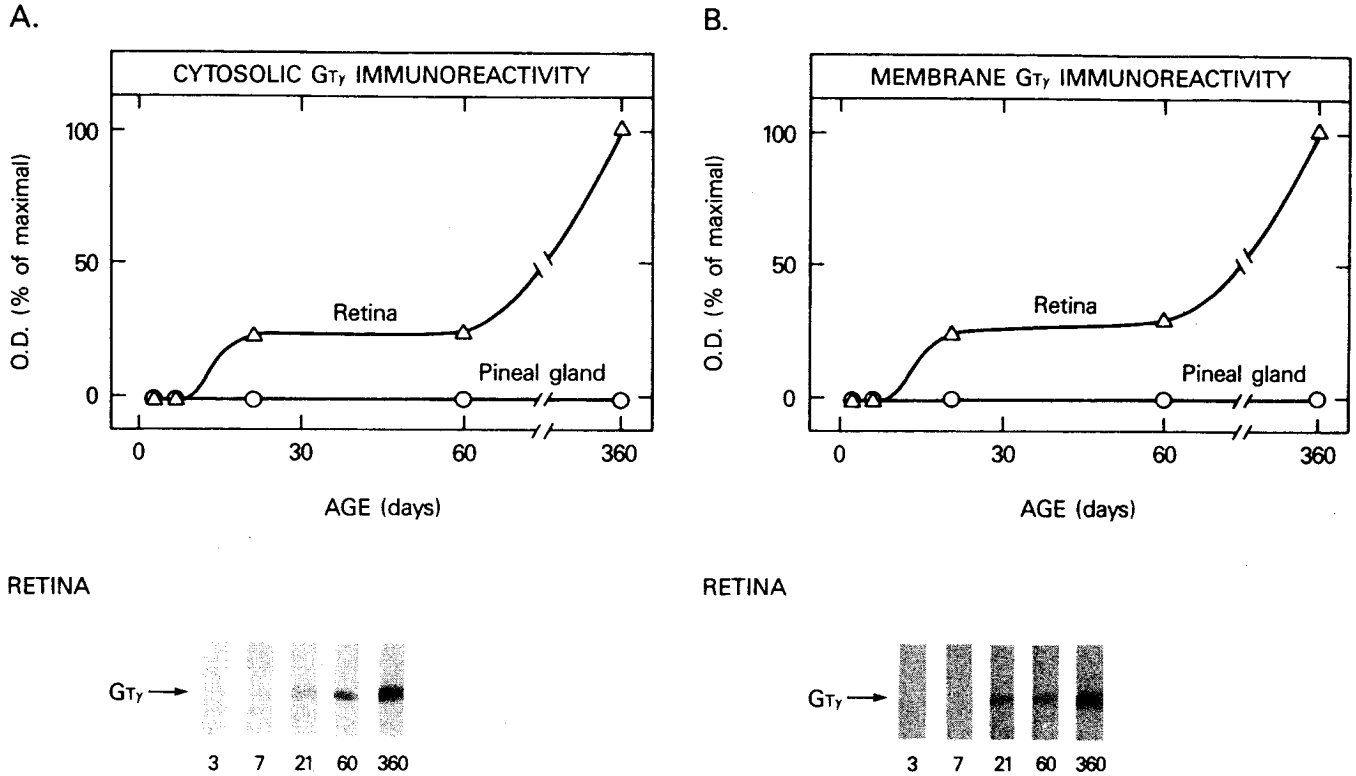
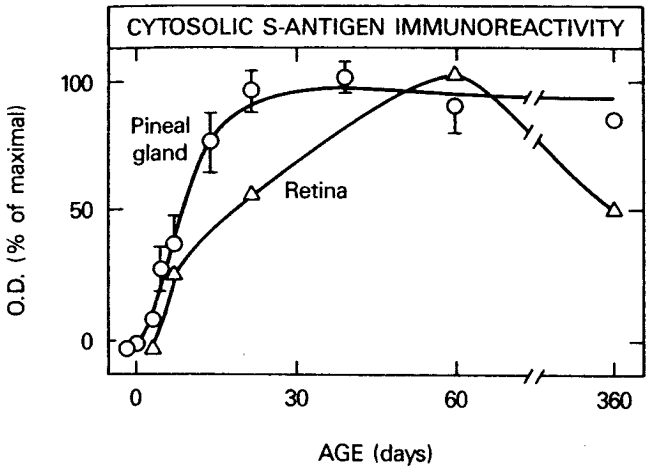


Fig. 3. Developmental profile GTγ in cytosol (A) and membrane (B). Pineal (80 μg) and retina (10 μg) cytosolic preparations (left panel) or membrane preparations (right panel) 80 μg and 10 μg, respectively from rats at different developmental stages were analyzed. GTγ was detected using rabbit anti-GTγ serum (PE[31]; 1:200) as described in Materials and Methods. Normalized data are presented above; the 100% value is the highest value for each tissue. Photographs of typical immunoreactive bands are presented below. Quantitation was performed as described in Materials and Methods. The pineal data are based on material prepared from five tissue pools obtained in three experiments; each time point represents the mean of the results of the analysis of each pool. Data are presented as the mean of % of maximum O.D. ± S.E. The absence of a S.E. bar indicates the error fell within the area of the symbol. The retinal data are based on two pools of retinae obtained from one experiment; data are presented as the average of the % of maximal value in each experiment (values were within 20%). For further details see Materials and Methods.

retina cytosol contains twice as much MEKA and 60 times more Gβ compared to the adult pineal cytosol (Table I). The remarkable difference in the amount of Gβ relative to the amount of MEKA probably reflects the fact that the GTGβ complex is relatively weakly associated with the plasma membrane[20], and that a major fraction of Gβ in the cytoplasm is probably complexed with GTα.

The second issue which stimulated this study was the function of the cytoplasmic MEKA/Gβγ complex. It is clear that it is not essential for cyclic AMP stimulation of pineal arylalkylamine *N*-acetyltransferase, the central element in pineal signal transduction; because this can be easily demonstrated prior to birth in the rat pineal gland[38], before MEKA is present. Whereas this suggests that MEKA and the MEKA/Gβ complex are not required for stimulation of arylalkylamine *N*-acetyltransferase, it does not exclude the possibility that cytosolic Gβ might influence arylalkylamine *N*-acetyltransferase activity in some way.

There is growing indication that Gβγ may play a direct role in signal transduction. For example, Gβγ can activate a K^+ channel[19], inhibit muscarinic stimulation of phospholipase C[21], inhibit calmodulin-stimulated adenylylcyclase[10] and activate phospholipase A_2 (for review, see Burch[7]). Perhaps the MEKA/Gβγ complex mediates a related cytosolic function in the pineal gland and retina which is regulated by cyclic AMP acting via cyclic AMP dependent protein kinase. Accordingly, it is reasonable to suspect that cytoplasmic Gβγ might influence pineal arylalkylamine *N*-acetyltransferase activity prior to the appearance of MEKA, and that the appearance of MEKA and association of MEKA with Gβγ places Gβγ under the control of cyclic AMP via cyclic AMP-dependent protein kinase. Interestingly, early in development the day-time arylalkylamine *N*-acetyltransferase activity is relatively high compared to that at later stages[9]. Although this has been attributed to the effects of circulating catecholamines, which seem to act as hormones controlling the pineal gland early in life[38], this view might not be entirely correct. Perhaps cytoplasmic Gβγ is in part responsible for these high levels of pineal arylalkylamine *N*-acetyltransferase activity.



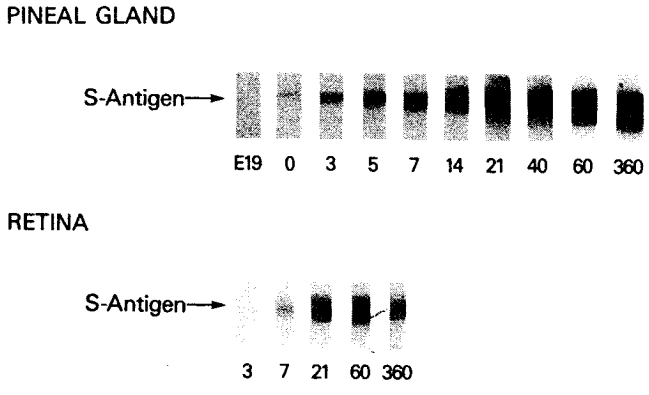


Fig. 4. Developmental profile of S-antigen. Pineal (10 μg) and retina (5 μg) cytosolic preparations from rats at various developmental stages were analyzed. S-Antigen was detected using a rabbit anti-bovine S-antigen serum (NEI04111083, 1:5000) as described in Materials and Methods. Normalized data are presented above; the 100% value is the highest value for each tissue. Photographs of typical immunoreactive bands are presented below. Quantitation was performed as described in Materials and Methods. The pineal data are based on material prepared from five tissue pools obtained in three experiments; each time point represents the mean of the results of the analysis of each pool. Data are presented as the mean of % of maximum O.D. $\pm$ S.E. The absence of a S.E. bar indicates the error fell within the area of the symbol. The retinal data are based on two pools of retinae obtained from one experiment; data are presented as the average of the % of maximal value in each experiment (values were within 20%). For further details see Materials and Methods.

Several interesting observations in this report deserve brief comment. One is that membrane associated Gβ in the pineal is present at relatively constant levels throughout development whereas retinal membrane Gβ appeared at day 21. This seems to be associated with the capacity to support functional signal transduction. For example, it has been noted that pineal adenylylcyclase activity is present early in life and it is possible to stimulate cyclic AMP production in the fetal pineal gland by treatment with cholera toxin[4]. This result is coordinated with the appearance of β- and α_1-adrenergic receptors[4,33]. In contrast, the retina does not appear to be photoreceptive until after birth[2,36]

A second observation worthy of comment is that developmental profiles of rat pineal MEKA and of retinal MEKA in mice exhibiting retinal degeneration[14,15] are similar, with decreases occurring following early peaks. This may reflect the disappearance of phototransduction related processes in both tissues. There is a gradual decrease in rhythm in arylalkylamine *N*-acetyltransferase activity and melatonin in the pineal gland with age[27]. This might be related to the loss of MEKA, and it would be of interest to determine if retinal arylalkylamine *N*-acetyltransferase activity changes with age in both normal mice and mice with retinal degeneration.

Finally, the results of this study provide further indication of marked differences in the expression of members of the pineal/retinal subset of proteins during development (Figs. 1–4). These differences appear to reflect both tissue and protein specific mechanisms. The mechanisms controlling the developmental appearance of pineal/retinal proteins has not been elucidated. Hence, it might be useful to consider a working hypothesis describing pineal and retinal differentiation in which the expression of all pineal/retinal proteins is controlled by a single switch, and that downstream switches determine whether the proteins are expressed as a cassette of transmembrane signal transduction proteins, of intracellular signal transduction proteins, or of melatonin synthesizing proteins. Additional down-stream factors would determine the tissue specific expression, as described here and elsewhere. Investigations leading to a resolution of this process are now underway. Recent investigations have revealed the presence in the S-antigen gene of a unique regulatory sequence[37]. This sequence could be the key element shared by all pineal/retinal signal transduction proteins, or only with a cassette of intracellular signal transduction proteins. It seems reasonable to expect that this line of investigation will be extended to include all pineal/retinal proteins, resulting in a thorough understanding of the factors regulating their expression.

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