PURINERGIC MODULATION IN THE DEVELOPMENT OF THE RAT UNCROSSED RETINOTECTAL PATHWAY


Programa de Neurociências, Departamento de Neurobiologia, Instituto de Biologia, Universidade Federal Fluminense, Caixa Postal 100180, Niterói, CEP 24001-970, RJ, Brazil

Abstract—Adenosine is a neuromodulator implicated in nervous system development and plasticity and its effects are mediated by inhibitory (A1, A3) and excitatory (A2a, A2b) receptors. The role of adenosine in the synaptic activity depends mainly on a balanced activation of A1 and A2a receptors, which are activated by various ranges of adenosine concentrations. Herein, we investigated the expression of A1 and A2a receptors and also the accumulation of cAMP in the superior colliculus at different stages of development. Furthermore, we examined the effects of an acute in vivo blockade of adenosine deaminase during the critical period when the elimination of misplaced axons/terminals takes place with a simultaneous fine tuning of terminal arbors into appropriate terminal zones. Lister Hooded rats ranging from postnatal days (PND) 0–70 were used for ontogeny studies. Our results indicate that A1 expression in the visual layers of the superior colliculus is higher until PND 28, while A2a expression increases after PND 28 in a complementary developmental pattern. Accordingly, the incubation of collicular slices with 5'-N-ethylcarboxamido-adenosine, a non-specific adenosine receptor agonist, showed a significant reduction in cAMP accumulation at PND 14 and an increase in adults. For the anatomical studies, the uncrossed retinotectal projections were traced after the intraocular injection of horseradish peroxidase. One group received daily injections of an adenosine deaminase inhibitor (erythro-9(2-hydroxy-3-nonyl) adenine), 10 mg/kg i.p.) between PND 10 and 13, while control groups were treated with vehicle injections (NaCl 0.9%, i.p.). We found that a short-term blockade of adenosine deaminase during the second postnatal week induced an expansion of retinotectal terminal fields in the rostrocaudal axis of the tectum. Taken together, the results suggest that a balance of purinergic A1 and A2a receptors through cAMP signaling plays a pivotal role during the development of topographic order in the retinotectal pathway. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: critical period, adenosine receptors, development, superior colliculus, cAMP, topographic order.

Adenosine is an endogenous modulator in the mammalian CNS implicated in neuroprotection and synaptic plasticity. Two main sources of extracellular adenosine are known: adenosine released by bi-directional nucleotide transporters or the extracellular conversion of adenosine nucleotides by ectonucleotidases (Latini and Pedata, 2001). The effects of adenosine are mediated by metabotropic receptors classified as A1, A2a, A2b, and A3, which are coupled to adenyl cyclase (Wardas, 2002; Fredholm et al., 2005a). The neuromodulatory role of adenosine in the synaptic activity depends mainly on a balanced activation of inhibitory A1 receptors and facilitatory A2a receptors which are activated by various ranges of adenosine concentrations (Correia-de-Sa et al., 1996). Despite the classic inhibitory actions of adenosine, many reports have indicated that adenosine has excitatory effects in the hippocampus (Nishimura et al., 1990; Okada et al., 1990; Sebastiao and Ribeiro, 1992; Rebol et al., 2003) and superior colliculus (SC). This effect is mediated by cAMP signaling through A2a receptors (Okada et al., 1990).

The rodent uncrossed retinotectal pathway is a useful model to study the developmental mechanisms involved in the establishment of synaptic specificity. This projection undergoes an extensive reorganization within the first two/three postnatal weeks when topographic distribution of terminal axons acquires an adult-like pattern (Land and Lund, 1979; Jeffery, 1984). Several lines of evidence have highlighted the role of cAMP on development and plasticity (Huang et al., 1994; Rodger et al., 2005). Indeed, cAMP signaling mediates the activity-dependent segregation of ipsilateral and contralateral retinal axons in the dorsal lateral geniculate nucleus (dLGN) and axonal elimination in the SC (Stellwagen and Shatz, 2002; Ravary et al., 2003).

The aim of this work was to investigate the role of purinergic system in the developing retinotectal pathway, by analyzing: (i) the ontogenesis of A1 and A2a receptors in homogenates of the SC, (ii) the levels of cAMP in slices of SC and the modulation by a non-selective adenosine receptor-agonist, and (iii) the effect of an acute blockade of adenosine deaminase during development of the uncrossed retinotectal topography. We found that A1 expression is higher within the critical period, while A2a expression is more evident after the fourth postnatal week. These data were also correlated with cAMP accumulation in the SC after the incubation with a purinergic agonist. Furthermore, a short-term blockade of adenosine deaminase during the second postnatal week induced a disruption of the retinotectal topography.
EXPERIMENTAL PROCEDURES

Lister Hooded rats were used for biochemical and neuroanatomical experiments. All animal use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local animal care committee. Studies were designed to minimize the number of animals used and their pain and suffering.

Western blot analysis

For the biochemical assays, rats at different postnatal ages were deeply anesthetized with a mixture of ether and distilled water (1:1) and decapitated. SC were dissected and homogenized in ice-cold 50 mM Tris–HCl pH 7.4. The resulting homogenates were centrifuged at 15,000 × g for 2 min at 4 °C. The pellet was resuspended in sample buffer containing sodium dodecyl sulfate (SDS). The analysis of adenosine receptor expression was carried out in total membranes of the SC. Protein concentration was measured by the method of Bradford (1976). Samples were separated by SDS/polyacrylamide gel electrophoresis (10% with a 4% concentrating gel) and electrotransferred to PVDF (polyvinylidene difluoride membranes, Amersham Biosciences, Piscataway, NJ, USA). After blocking for 2 h at room temperature with 5% defatted milk in Tris-buffered saline, pH 7.6 containing 0.1% Tween 20 (TBS-T), the membranes were incubated overnight at 4 °C with either a rabbit anti-adenosine A1 receptor A1:1000 dilution, from Oncogene, Calbiochem, San Diego, CA, USA) or rabbit anti-adenosine A2a receptor antibodies (1:2500 dilution, from Chemicon, Millipore Corporate, Billerica, MA, USA). After washing with TBS-T (3 × 10 min), the membranes were incubated with anti-rabbit secondary antibody (1:5000 dilution, Amersharn Biosciences, Piscataway, NJ, USA) in TBS-T during 1 h at room temperature. After two washing periods of 10 min in TBS-T and TBS, the membranes were incubated with enhanced chemiluminescence plus chemiluminescent kit (Amersham Biosciences, Piscataway, NJ, USA) during 5 min. After capturing the bands on hyperfilm (Amersham Biosciences, Piscataway, NJ, USA), the membranes were re-probed and tested for actin immunoreactivity to confirm that similar amounts of protein were applied into gels. Briefly, after the first exposure, membranes were washed with TBS and incubated with 0.2 M glycine pH 2.2 for 30 min at orbital rotation. Membranes were then blocked as described earlier and incubated with goat anti-β-actin antibody overnight (1:300, Santa Cruz, CA, USA). Then, membranes were washed and incubated with anti-goat secondary antibody (1:3000, Santa Cruz, CA, USA) and developed as described above. The densitometry analysis was performed using Gelplot analysis macro in ScionImage software, version 4.03 (Scion Corporation, Frederick, MD, USA). The integrated density of A1 or A2a receptor bands was normalized against β-actin labeling in each corresponding gel for each age studied.

cAMP accumulation

SC were dissected in ice cold Eagle modified-culture medium buffered with 20 mM HEPES, pH 7.3. Then, slices of SC (200 mg of protein/ml) were pre-incubated for 5 min at 37 °C in the same solution with 10 nM RO201724, a phosphodiesterase inhibitor. Afterwards, samples were incubated in the presence or absence of 5’-N-ethylcarboxamido-adenosine (NECA; 0.1 mM final concentration) for 15 min. The reaction was interrupted by the addition of trichloroacetic acid to a final concentration of 5%, and the tissue was frozen immediately until use. After centrifugation at 15,000 × g for 30 min, the supernatant was passed through an ion exchange resin column (Dowex 50) to remove the trichloroacetic acid and other nucleotides (Matsuzawa and Nirenberg, 1975). The samples containing cyclic AMP were assayed by the method of Gilman (1970). The protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin (BSA, Sigma Co., St. Louis, MO, USA) as a standard. cAMP levels were expressed in picomoles per milligram of protein. The values presented are mean ± SEM of at least three different experiments (performed in duplicate).

In vivo study

The animals received daily i.p. injections of erythro-9(2-hydroxyl-3-onyl) adenosine (EHNA, 10 mg/kg ip, Sigma Co., St. Louis, MO, USA) from PND 10 to PND 13. Control matched-groups received vehicle injections (NaCl 0.9% i.p.). After appropriate survival, animals received, under anesthesia (ether/water 1:1), an intracocular injection of 5 μl of a solution of 30% horseradish peroxidase (HRP, Sigma, type VI, Sigma Co., St. Louis, MO, USA) in 2% dimethylsulfoxide in NaCl 0.9% in the right eye to label the uncrossed retinotectal pathway. After 24 h, the animals were deeply anesthetized with an overdose of anesthetic and transcardially perfused with saline NaCl 0.9% followed by a mixture of 1% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer pH 7.2. The brains were dissected and cryoprotected overnight in a solution of 20% sucrose in the same buffer. Alternate 40 μm parasagittal sections were incubated free floating with tetramethylbenzidine (TMB, Sigma Co., St. Louis, MO, USA) as the chromogen (Mesulam, 1978). Sections were rapidly dehydrated in ethanol and cleared in xylene, and the coverslips mounted with Entellan (Merck). Sections were analyzed using dark-field optics in a Zeiss microscope.

Neuroanatomical analysis

Retinotectal terminal labeling was compared between groups in every section through the SC. For all experimental groups, the anterograde labeling to the contralateral SC was carefully examined in the whole rostrocaudal axis and taken as an internal control for homogeneous tracer uptake, transport, histochemical labeling or any lesion due to the tracer delivery procedure. The anterograde transport of HRP using TMB histochemistry reveals two patterns of labeling in the SC: (1) terminal labeling, characterized by dense and non-oriented deposits of TMB reaction product observed within clusters; (2) fiber labeling, characterized by discrete deposits of TMB along axons, giving an oriented bias to the TMB labeling. In the SC, clusters represent the convergence of axonal arbors within target zones whereas fiber labeling is mostly found in their surroundings, along the ventral and anterior most regions, where axons are en route to their targets. Since terminals and fibers are easily distinguishable under dark field optics, clusters were defined as spots of high density of terminal labeling where non-oriented, saturating deposits of TMB reaction product were found (see inset Fig. 5). As the crossed retinotectal projection should label the whole extension of the collicular visual layers we discarded animals in which the TMB reaction product revealed partial or suboptimal labeling of any sector of the contralateral colliculus. We also discarded animals in which the pattern of TMB reaction did not allow a clear discrimination between terminal and fiber labeling. Microscope images were captured using a digital camera (Sound Vision SV Micro) mounted to the microscope stage, and quantitative analysis of terminal labeling densities was carried out using Scion Image software (Scion Image Beta 4.03, Scion Corporation). Therefore we analyzed in TIFF images the distribution of the uncrossed retinotectal terminal pattern of TMB reaction product were found (see inset Fig. 5). As the crossed retinotectal projection should label the whole extension of the collicular visual layers we discarded animals in which the TMB reaction product revealed partial or suboptimal labeling of any sector of the contralateral colliculus. We also discarded animals in which the pattern of TMB reaction did not allow a clear discrimination between terminal and fiber labeling. Microscope images were captured using a digital camera (Sound Vision SV Micro) mounted to the microscope stage, and quantitative analysis of terminal labeling densities was carried out using Scion Image software (Scion Image Beta 4.03, Scion Corporation). Therefore we analyzed in TIFF images the distribution of the uncrossed retinotectal terminal labeling. Clusters areas were measured as the highest density of terminal labeling located in the rostral aspect of the colliculus. Global pixel density was used to analyze both the distribution of scattered fibers throughout the SC and terminal fields located within clusters. Pixel densities were measured on a 0–255 scale, in which 255 corresponded to white. Background...
densities obtained in deep non-visual layers of the colliculus were subtracted from values in the superficial layers in every section. Samples were taken for at least three/four consecutive sections per animal, starting at 160 μm from the midline.

Statistics

Graphs were plotted using Prism 3.0 software and the statistical analysis was performed using one-way ANOVA with Tukey post test or Student’s t-test and differences were considered significant when P<0.05.

RESULTS

Ontogenesis of purinergic receptors in the SC

The expression of adenosine receptors has been characterized in different brain areas (Sebastiao and Ribeiro, 1996; Ochiishi et al., 1999; Fredholm et al., 2005b). Functional evidence for the presence of A1 and A2a adenosine receptors has been shown in the avian optic tectum (Ventura and Paes de Carvalho, 1987). However, the ontogenesis of these receptor subtypes in the mammalian SC is unknown. We performed Western blots of protein extracts from the SC with polyclonal antibodies against A1 and A2a receptors. Blots were stripped and incubated with β-actin antibody to control protein loading. The ontogenesis of adenosine A1 receptor is shown in Fig. 1A. The antibody detected a single band of 39 kDa. Quantitative analysis revealed that the A1 receptor presents maximal expression in early stages of development, followed by a reduction after the fourth postnatal week (Fig. 1B).

The pattern of A2a receptor expression is shown in Fig. 2A. A single band of 44 kDa was detected in all ages studied and β-actin expression was identical as those described above. Immunoblot analysis revealed that A2a protein presents low levels between postnatal days (PND) 0 and 14. Afterwards, maximal immunoreactivity was observed after the end of the critical period of development (Fig. 2B).

Fig. 1. Expression of adenosine A1 receptor in the development of retinotectal pathway. (A) Representative Western blot. SC proteins were resolved by SDS/polyacrylamide gel electrophoresis, following incubation with an anti-A1R antibody (top panel). Blots were then reprobed with a β-actin antibody (bottom panel). Proteins were visualized using enhanced chemiluminescence (ECL). (B) Quantification of adenosine A1 receptor levels. ECL signals corresponding to the A1R were quantified by densitometry. Data are from five independent determinations and are shown as mean±SEM of four (PND 0), four (PND 7), four (PND 14), four (PND 28), three (PND 42), or three (PND 70) animals. Asterisks represent a significant difference between groups * P<0.05.

Fig. 2. Expression of adenosine A2a receptor in the development of retinotectal pathway. (A) Representative Western blot. SC proteins were resolved by SDS/polyacrylamide gel electrophoresis, following incubation with an anti-A2aR antibody (top panel). Blots were then reprobed with a β-actin antibody (bottom panel). Proteins were visualized using enhanced chemiluminescence (ECL). (B) Quantification of adenosine A2a receptor levels. ECL signals corresponding to the A2aR were quantified by densitometry. Data are from three independent determinations and are shown as mean±SEM of three animals for each point. Asterisks represent a significant difference between groups ** P<0.01.

CAMP accumulation during development

Previous studies have shown that CAMP is involved in different aspects of CNS development, including neuronal survival, axon outgrowth/guidance and synaptic stabilization (Crump et al., 2001; Rodger et al., 2005; Argaw et al., 2008). Therefore, we analyzed CAMP accumulation in slices of SC at different developmental stages. During early stages, basal cAMP levels were lower (PND 0 = 229.6±47.6; PND 7 = 324.9±41.0), followed by a significant increase at PND 14 (1925.3±400.9), soon after eye opening. Levels of basal cAMP remained higher be-
between the fourth and sixth postnatal weeks (PND 28 = 1460.4 ± 568.9; PND 42 = 1052.4 ± 278.2) followed by a decay in adulthood (PND 70 = 438.3 ± 144.6) (Fig. 3).

As the expression of A₁ and A₂a receptors was predominant during and after the critical period, respectively, we tested whether the expression of purinergic receptors correlates with their activities in the SC. For that purpose we chose a non-specific adenosinergic agonist (NECA). At PND 14, 0.1 mM NECA induced in SC slices a decrease in cAMP accumulation (59.8% of control), whereas at PND 42, the adenosine agonist induced a significant increase in cAMP accumulation relative to control, non-stimulated slices (153.4% of control) (Fig. 4).

**Effect of adenosine deaminase blockade in the early development of the uncrossed retinotectal pathway**

As described previously, the retinal input from both eyes overlaps in the visual targets during early stages of postnatal development when uncrossed retinal axons are eliminated from the caudal and subpial aspects of the colliculus, while a set of topographically correct axon terminals progressively condenses in clusters located at the stratum griseum superficiale (SGS)/SO border of the rostral colliculus (Land and Lund, 1979; Serfaty and Linden, 1994; Serfaty et al., 2005). To test the hypothesis that adenosine exerts a neuromodulatory effect on the development of retinotectal circuitry, a group of animals was treated with either adenosine deaminase inhibitor (EHNA, 10 mg/kg) or vehicle (saline 0.9%) between PND 10 and 13. The topographic organization of the uncrossed pathway of vehicle and EHNA-treated rats was analyzed in the medial (Fig. 5A, B, respectively) and lateral (Fig. 5C, D, respectively) aspects of the colliculus. In vehicle-treated animals, the ipsilateral retinotectal projection showed, at PND 14, an adult-like pattern with a dense and well-defined terminal zone located at the ventral border of the SGS in the anterior third of the colliculus (Fig. 5A, C). On the other hand, the short-term treatment with EHNA induced an expansion of uncrossed projection, relative to the control pattern. In this group, we observed an increase in HRP terminal/fiber labeling not only in rostral SGS but also throughout the dorsal and caudal aspects of the SC (Fig. 5B, D). This indicates an active reorganization of the retinotectal axons induced by increased levels of endogenous adenosine. Quantitative analysis showed that EHNA-treated rats presented expanded clusters of HRP terminal labeling both in the medial and lateral aspects of the colliculus as compared to vehicle-matched groups (Fig. 6A). Moreover, EHNA treatment induced an increase in innervation density throughout the rostrocaudal axis for both the medial and the lateral sections of the SC (Fig. 6B, C).

**DISCUSSION**

The development of the sensory systems involves crucial steps that are fundamental to the appropriate processing of information. Among those, axonal elimination and extensive rearrangements of synaptic contacts generate topographically organized projections. Adenosine is an important neuromodulator that controls synaptic transmission and plasticity, among other processes. Herein, we demonstrated the expression of A₁ and A₂a adenosine receptors and the modulation of cAMP levels through a purinergic agonist during the development of the SC. Furthermore, the pharmacological increase in endogenous adenosine levels within the critical period resulted in anatomical reorganization of the retinotectal pathway with expansion of terminal fields. These data suggest a role for adenosine in the development of retinotectal topography.

**Purinergic system in the SC**

The present study demonstrates that A₁ and A₂a receptor expression changes in the SC, according to the stage of development. Performing Western blot analysis of the receptor density we showed that the expression of A₁ receptors is higher until the fourth postnatal week, while A₂a receptors are more evident after this period (Figs. 1, 2). This is in agreement with binding studies in hippocampus and cortex (Cunha et al., 1995). In addition, the reported low A₁ receptor density observed in the hippocampus of elderly animals was correlated to a decrease in the agonist ability to inhibit neurotransmission (Sebastiao et al., 2000).

---

**Fig. 3.** Accumulation of cAMP in the development of SC. Data are from five independent determinations and are shown as mean ± SEM of nine (PND 0), three (PND 7), three (PND 14), three (PND 28), six (PND 42), or eight (PND 70) animals. Asterisks represent a significant difference from PND 0. ** P < 0.01; *** P < 0.001.

**Fig. 4.** Accumulation of cAMP in slices of SC after NECA incubation at PND 14 or PND 42. Data are from five independent determinations and are shown as mean ± SEM of three (PND 14), or five (PND 42) animals. Asterisks represent a significant difference from control, non-treated matched groups. * P < 0.01.
Concerning A2a receptors, it has been shown that it presents higher activity in old hippocampus which could be correlated not only to a higher receptor expression but also to an increase in G protein coupling (Rebola et al., 2003). It has been shown in the hippocampus and cortex that A2a activation leads to a reduction in agonist activation of A1 receptor, suggesting that A2a receptor is involved in down-regulation of A1 receptor (Lopes et al., 1999, 2002; Rebola et al., 2005). The complementary pattern of adenosine receptor expression found herein during the development of retinocollicular connections was also in conformity with previously published data which point out opposite effects for these receptors in the regulation of synaptic transmission: A1 receptor has been associated with a decrease in neurotransmitter release and hyperpolarization of postsynaptic cells, while A2a receptor has been reported to induce facilitation in neurotransmitter release and synaptic transmission (Cunha, 2001). Therefore, the temporal pattern of A1 and A2a activation could be respectively associated with intense synaptic reorganization during early development and with synaptic stabilization observed at the end of the critical period.

Adenosine receptors are coupled to different second messengers pathways, among them cAMP signaling. Cyclic AMP–mediated processes have been described to modulate development and plasticity in the CNS (Huang et al., 1994; Wang et al., 2004) being involved in retinal ganglion cells survival and regeneration (Meyer-Franke et al., 1995, 1998; Watanabe et al., 2003; Argaw et al., 2008), axonal guidance (Rodger et al., 2005), and as a presynaptic mediator of mossy fiber long-term potentiation in the hippocampus (Frey et al., 1993; Huang et al., 1994). Thus, we decided to investigate whether cAMP levels correlate with the pattern of adenosine receptor expression. Biochemical assays showed a low level of basal cAMP during the first postnatal week, followed by a significant increase soon after eye opening (at PND 14). Basal cAMP remained higher between the fourth and sixth postnatal weeks and decayed in adulthood (Fig. 3). This profile is also consistent with a role for cyclic-AMP in different aspects of retinotectal development such as ephrin-dependent retraction of retinotectal transient axons as well as a refinement process which leads to retinotopic map formation in mice (Ravary et al., 2003; Plas et al., 2004; Nicol et al., 2006). After eye opening at PND 14, cAMP-driven synaptic stabilization would be maximal compared to an unstable pattern found in early development. A question remains as to which mechanisms would be responsible for synaptic maintenance in adulthood when cAMP levels decay: a possible explanation would be related to a signaling cascade triggered during development by cAMP, leading to PKA-CREB phosphorylation, in a process reminiscent of the late long-term potentiation at hippocampal synapses (Barco et al., 2002). Alternatively, synaptic maintenance in adults could be achieved by other signaling molecules such as arachidonic acid metabolites (Campello-Costa et al., 2006).

There are many neurotransmitters cooperating to regulate cyclic AMP levels in the SC (Ishikawa et al., 1997; Gonzalez et al., 2008). Thus in the present study, we investigated cAMP accumulation via a selective activation of adenosine receptors. Slices of the SC were incubated with a non-specific adenosinergic agonist during and after the critical period of retinotectal development, when A1 and A2a receptors are respectively predominant. Our data showed that NECA treatment produced a decrease in cAMP accumulation at PND 14 and an opposite effect at PND 42. These results point out that differential expression of A1 and A2a adenosine receptors reflects their modulatory activity on cAMP modulation during development of visual pathways.

**In vivo blockade of adenosine deaminase activity: role of adenosine in the retinotectal topography**

The development of topographic order in the mammalian subcortical visual pathways has so far been considered to be completed at the end of the critical period which closes...
around the third postnatal week in rodents. There are many mechanisms cooperating in the development of topographic order in the visual system, including AMPA/NMDA receptor balance (Taylor et al., 2005), and also local messengers such as nitric oxide and arachidonic acid (Campello-Costa et al., 2000, 2006). Also the role of 5-HT in the development and plasticity of retinocollicular pathways has been demonstrated through pharmacological and nutritional approaches (Bastos et al., 1999; Gonzalez et al., 2008; Penedo et al., 2009). In the cerebral cortex, GABA is also an important molecule that guides the critical period for use-dependent formation of ocular dominance plasticity (Hensch and Fagiolini, 2005). Indeed, all of the mechanisms described above could be acting in a synergistic way to promote synaptic stabilization in the most coincident afferents. Additionally, adenosine has been described as a neuromodulator in almost every classic neurotransmitter system including glutamate, 5-HT, GABA, among others (Fredholm et al., 2005a,b). In the glutamatergic system it has a well-known role regulating both neurotransmitter release and post-synaptic currents according to adenosine receptors recruitment (Tebano et al., 2005; Ciruela et al., 2006).

In the current study, we investigated whether adenosine could also be involved in synaptic specificity of retinotectal circuits during the critical period. Systemic treatment with EHNA (10 mg/kg i.p.) has been shown to induce an increase in adenosine levels in the cerebrospinal fluid and also an increase in excitatory post-synaptic potentials in the SC in adult rats (Ishikawa et al., 1997). Hence, we evaluated a short-term adenosine deaminase inhibition, between PND 10 and PND 13, when A1 receptor expression is more evident, and the major wave of axonal elimination has already occurred (Jeffery, 1984; Serfaty and Linden, 1994). Our results show that accumulation of endogenous adenosine induced by EHNA treatment, just before eye opening, disrupts retinotectal topography (Fig. 5). Therefore, adenosine acting predominantly through A1 receptors could be acting as an inhibitory filter, allowing a less stable state of developing synapses and a Hebbian stabilization only at the most coincident synapses leading to topographic refinement. Indeed, purinergic receptor activation depends on the pattern of pre-synaptic activity: low frequency activity likely to occur in non-correlated synapses leads to preferential activation of A1 receptors, while high frequency activity elicits A2a receptor activation in correlated inputs (Cunha, 2008). This is in accordance with the current idea that the fine tuning of brain circuitry involves activity-dependent modifications of synaptic efficacy which are necessary for sculpting axonal terminal fields (Shatz, 1990; Katz and Shatz, 1996; Mrsic-Flogel et al., 2005).

The present results are also consistent with previous observation on the role of A1 receptors as a pre-synaptic inhibitory modulator both in normal and regenerating goldfish retinotectal transmission (Zhang and Schmidt, 1998, 1999) and extend the role of adenosine receptors to the development of mammalian retinotectal projections. In this scenario, a shift in purinergic signaling from inhibitory (A1) to excitatory (A2a) modulation of synaptic transmission may play an important role for the refinement and stabilization of central visual connections.

**CONCLUSIONS**

In conclusion, we present evidence that the enhancement of adenosine levels induced by the blockade of adenosine deaminase during the critical period of topographic refinement in the SC influences the fine tuning of topographic order in the visual system, including AMPA/NMDA receptor balance (Taylor et al., 2005), and also local messengers such as nitric oxide and arachidonic acid (Campello-Costa et al., 2000, 2006). Also the role of 5-HT in the development and plasticity of retinocollicular pathways has been demonstrated through pharmacological and nutritional approaches (Bastos et al., 1999; Gonzalez et al., 2008; Penedo et al., 2009). In the cerebral cortex,
maps and thus circuit formation. Its effects, probably, involve A₁ receptors which present a high expression during the first three postnatal weeks. A₂A receptor is also expressed in the SC being more evident after the fourth postnatal week, suggesting its participation in maintenance of retinotectal axons.

Acknowledgments—We thank Mrs. Maria da Conceição Paiva Silva and Maria Leite Eduardo Pontes for technical support; Mr. Bernardino Matheus-dos-Santos and Alejandro de Jesus Re-sende for animal care. This work was supported by grants from the Brazilian National Research Council—CNPq, Research Foundation of the State of Rio de Janeiro-FAPERJ, CAPES and PRONEX/MCT, PROPP-UFF.

REFERENCES


Huang YY, Li XC, Kandel ER (1994) cAMP contributes to mossy fiber LTP by initiating both a covalently mediated early phase and macromolecular synthesis-dependent late phase. Cell 79:69–79.


(Accepted 15 July 2009)
(Available online 18 July 2009)