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Neuroscience Research 42 (2002) 309–316

Neuroscience Research

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Neuronal expression of arylalkylamine *N*-acetyltransferase (AANAT) mRNA in the rat brain

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Received 13 December 2001; accepted 29 January 2002

Abstract

The role of arylalkylamine *N*-acetyltransferase (AANAT) in neuronal functioning has been suggested based on biochemical assays; only scarce evidence indicates neuronal expression of the mRNA encoding for this enzyme that catalyzes the conversion of serotonin into *N*-acetylserotonin. Using a quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay with internal standards, and an in-situ RT-PCR hybridization assay we found evidence for the expression of AANAT in the rat brain. In the localization studies, the most prominent AANAT mRNA signal was found in the granule neurons of the hippocampus, the olfactory bulb, and the cerebellum, and in the gray matter of the spinal cord. Diurnal differences in AANAT mRNA content were observed in the pineal gland but not in the hippocampus; the content of AANAT mRNA was lower both in the pineal gland and the hippocampus of old (24 months) compared with young (2 months) rats. These data are consistent with the hypothesis that AANAT may play a physiological role in mammalian central nervous system neurons. Further studies are warranted into the possible functional significance of neuronal expression of AANAT mRNA. © 2002 Published by Elsevier Science Ireland Ltd and the Japan Neuroscience Society.

Keywords: Neuronal arylalkylamine *N*-acetyltransferase (AANAT); Melatonin; Pineal; Diurnal; Serotonin, aging

1. Introduction

It is generally believed that serotonin *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase; AANAT; EC 2.3.1.87) is an enzyme that is almost exclusively expressed in the pineal gland and in the retina. This enzyme catalyzes the metabolism of the neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) into *N*-acetylserotonin (NAS) (Ferry et al., 2000) an intermediate in the synthesis of melatonin. Thus, the functional role of AANAT appears to be restricted to the endocrine and the visual systems. Nevertheless, in lower organisms, such as the fruit fly (*Drosophila melanogaster*), the localization of AANAT mRNA was found to be predominantly in the nervous system (Brodbeck et al., 1998; Hintermann et al., 1995, 1996) which suggests that in insects, NAS and/or melatonin possibly participates in the process of neuronal functioning/neurotransmission. Although the sequences of the *Drosophila* (Brodbeck et al., 1998; Hintermann et al., 1996) and the mammalian (Borjigin et al., 1995; Coon et al., 1995, 1996) AANAT genes do not show significant homology, there is biochemical evidence indicative of neuronal localization of NAS, and consequently also of neuronal AANAT, in the mammalian brain as well.

Evidence for the neuronal presence of NAS in the mammalian brain was first obtained using specific anti-NAS antibodies (Psarakis et al., 1982). These investigators found that in the rat hippocampus, NAS immunoreactivity was primarily present in the cell bodies in the granular cell layer of the dentate gyrus. They also noticed age-dependent changes in the expression of this immunoreactivity; it was detectable as early as 20 days postconception, and it increased with age, reaching adult levels 10 days later (Psarakis et al., 1982). With the use of a biochemical assay, evidence of AANAT enzymatic activity was also found in the rat brain (Chae et al., 1999; Paul et al., 1974). Although generally

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0168-0102/02/$ - see front matter © 2002 Published by Elsevier Science Ireland Ltd and the Japan Neuroscience Society.
PII: S0168-0102(02)00011-1

rather low, CNS AANAT activity was distributed heterogeneously, with higher levels found in the spinal cord, the olfactory bulb, the cerebellum, and the hippocampus (Gaudet et al., 1991). Additional studies with anti-NAS antibodies also indicated a heterogeneous neuronal distribution of NAS: in the granular layer of the cerebellum, the tractus spinalis nervi trigemini, and the reticular formation (Pulido et al., 1983b). These authors also noted that the pattern of NAS distribution was different from that of its precursor, serotonin.

Studies of the pineal gland have established that in most mammals, including the rat, the synthesis of NAS and melatonin is predominantly regulated by a circadian rhythm-sensitive, cAMP-dependent regulation of AANAT gene expression (Borjigin et al., 1995; Pfeffer and Stehle, 1998; Roseboom et al., 1996). For example, in the rat pineal, a greater than 150-fold nocturnal increase in AANAT mRNA content has been described (Roseboom et al., 1996) and attributed to noradrenaline acting through a cAMP mechanism. The AANAT gene promoter was recently analyzed using deletions and site-directed mutagenesis, and it was concluded that cAMP could activate the AANAT gene via a cAMP-responsive element (CRE) located within two helical turns of an inverted CCAAT box (Baler et al., 1997). Also, cerebellar NAS, which is mostly localized in the cell bodies of granule cells is known to be affected by a stimulation of adrenergic receptors. Treatment of rats with the β-adrenergic agonist isoproterenol resulted in a significant increase in cerebellar neuronal NAS immunoreactivity (Pulido et al., 1983a), which has been interpreted as evidence for upregulated NAS synthesis in the rat brain. Direct evidence for β-adrenergic stimulation of brain AANAT activity has also been provided (Friedhoff and Miller, 1977).

Depending on the method used, investigations of the presence of AANAT mRNA in the rat brain have concluded that there is either no brain AANAT mRNA (Northern blot assay) (Borjigin et al., 1995; Roseboom et al., 1996), or that its levels are very low [polymerase chain reaction (PCR) assay] (Roseboom et al., 1996; Stefulj et al., 2001; Uz and Manev, 1999), or that it is expressed only in certain brain regions (Hamada et al., 1999). To clarify whether, indeed, AANAT mRNA is expressed in neurons, we performed experiments described in this report.

2. Material and methods

Male pigmented Brown–Norway (BNSSN, colonies #217 and #218) rats and albino Sprague–Dawley and Fisher 344 rats were purchased from Harlan (Indianapolis, Indiana). Most experiments were performed on 2-month-old rats. When indicated, we used 24-month-old rats or 7-day-old rat pups. The animals were housed under conditions of controlled temperature (23 ± 2 °C) and illumination (14-h light:10-h darkness; darkness commenced at 18:00 h). The protocol for the use of animals was approved by the University Animal Care Committee.

Quantitative reverse-transcription/polymerase chain reaction (RT-PCR) assays of AANAT and cyclophilin (cyc) mRNAs were performed as described in detail elsewhere (Uz and Manev, 1999). Briefly, the tissue (brain regions, pineal glands, or a pool of six to seven culture dishes) was processed for RNA isolation as described by Chirgwin et al. (1979). After each extraction, to check for possible DNA contamination, one sample was run by RT-PCR without adding reverse transcriptase enzyme. The oligonucleotide primer pairs were designed to allow amplification of 51–493 bp for AANAT (5′: TGA GCG CGA AGC CTT TAT CTC AGT; 3′: TGT GGC ACC GTA AGG AAC ATT GCA) (Coon et al., 1995) and of 177–474 bp for cyclophilin (5′: ATT TGG CTA TAA GGG TTC CTC; 3′: ACG CTC CAT GGC TTC CAC AAT) (Danielson et al., 1988). Each primer pair contained a comparable G/C content to minimize variability in hybridization efficiency at the annealing temperature. The specificity of the AANAT and the cyclophilin products was confirmed by sequencing the amplified areas using the Sequenase Version 2.0 DNA Sequencing Kit (USB/Amersham). Internal standard templates were generated by site-directed mutagenesis using PCR overlap extension (Uz and Manev, 1999). The total RNA (1 μg) and decreasing concentrations of cRNA (i.e. the AANAT internal standard) were reverse transcribed and PCR amplified using the specific primers. The RNA/cRNA mixtures were denaturated at 80 °C for 6 min and then reverse transcribed with cloned Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Gibco, BRL; 200 U) in RT buffer containing 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 1 mM deoxynucleotide triphosphates (dNTPs) (Gibco, BRL) using random hexamers (Pharmacia Biotech; 5 mM) and ribonuclease inhibitor (HPRI) (Amersham; 28 U) in a volume of 20 μl. The RT mixture was incubated at 37 °C for 60 min to promote cDNA synthesis. The reaction was terminated by heating the samples at 98 °C for 5 min, and the mixture was quick-chilled on ice. As a control, in all assays one RT reaction was performed in the absence of RNA. Competitive PCR amplification: after termination of the RT reaction, cDNA aliquots containing reverse-transcribed material were amplified with Hot *Tub* DNA polymerase (Amersham) in the Thermal Cycler (Perkin–Elmer, 9600). The amplification mixture contained cDNA, 0.5 μM specific primer pairs, 200 μM dNTPs, 1.5 mM $MgCl_2$, 50 mM Tris–HCl (pH 9.0), 20 mM ammonium sulfate, 15 mM KCl, and 1.5 U of Hot *Tub* polymerase in a 100 μl

volume. Trace amounts of [^{32}P]dCTP (Amersham; 0.5–1 μCi/sample) were included during the PCR step for subsequent quantification. The PCR mixture was amplified for 30 cycles with denaturation (94 °C, 15 s), annealing (60 °C, 30 s), and elongation (72 °C, 30 s) amplification steps. The reaction was terminated with a 5-min final elongation step. Following amplification, aliquots were digested with *Bgl*II in triplicate and run by agarose gel electrophoresis. To quantitate the amount of the product corresponding to the reverse-transcribed and amplified mRNA, the ethidium bromide-stained bands were excised and the radioactivity determined by Cerenkov counting (Santi et al., 1994). The results are expressed as either attomoles of AANAT mRNA/μg total RNA, and/or as the ratio of AANAT/cyc mRNA. Statistical analysis was performed using the non-parametric Mann–Whitney *U*-test (significance was accepted at $P < 0.05$).

Qualitative in situ RT-PCR hybridization assay of AANAT mRNA localization and immunostaining were performed as follows. Rats were anesthetized with 50 mg/kg pentobarbital and perfused through the ascending aorta with 0.9% saline followed by 300 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) at a flow rate of 15 ml/min. The brain, the pineal glands, or the spinal cord were dissected and submerged in 20% sucrose/4% paraformaldehyde overnight at 4 °C for cryoprotection. The tissue was sectioned into coronal 25-μm slices using a microtome/cryostat. In the in-situ assay, we employed fixed, floating coronal sections stored in buffered 4% paraformaldehyde at 4 °C. In this condition, mRNA in the tissue is stable for at least 8 weeks. The sections were incubated with 0.1 M phosphate buffer saline (PBS), pH 7.4, for 15 min at room temperature, followed by an incubation with 1 μg/ml proteinase K in PBS-Tween for 20 min at 37 °C. The sections were incubated twice in 'glycine solution' (0.75 g glycine/100 ml of 0.1 M PBS, pH 7.4) for 5 min at room temperature and treated with 13% triethanolamine solution (pH 8.0) containing 2.5% acetic anhydride for 10 min at room temperature. In order to eliminate the amplification of genomic DNA the sections were treated with RNase-free DNase1 [1 U/ml in 40 mM Tris–HCl (pH 7.4), 6 mM $MgCl_2$, 2 mM $CaCl_2$] at 37 °C overnight. After the DNase digestion the sections were washed in Tris–EDTA buffer (pH 8.0) twice for 5 min each, and subsequently washed in PBS for 5 min. Thereafter, the free floating sections were placed in a thin wall 200 μl thermo-cycler tube (two sections per tube) containing 50 μl of RT-PCR reaction mixture consisting of 25 mM Tris (pH 8.3), 1.2 mM $MgCl_2$, 0.2 mM dNTP precursors, 0.2 μM AANAT anti-sense primer (TGT GGC ACC GTA AGG AAC ATT GCA), 0.2 μM AANAT sense primer (TGA GCG CGA AGC CTT TAT CTC AGT), 5 μCi alpha-^{33}P-dATP, and 1 μl of SuperScript II RT/Taq Mix (Gibco BRL, Gaithersburg, MD). Complementary DNA was synthesized with reverse transcriptase and amplified by Taq DNA polymerase in one step using the SuperScript ONE-STEP RT-PCR System (Life Technologies). The reverse transcription was performed at 50 °C for 1 h, and terminated at 94 °C for 2 min. Then, the PCR amplification was performed in 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min, followed by one cycle of final extension at 72 °C for 10 min. Negative controls for in situ RT-PCR were also used: (a) conditions in which the Taq DNA polymerase was omitted; (b) omitting the specific primers from the assay solutions; or (c) 2-h pre-digestion of tissue sections with RNase before RT. After the RT-PCR reaction, the sections were transferred to multi-well micro plates and washed in PBS twice for 15 min. Sections were mounted on glass slides, air dried and exposed to the Amersham β-max film for 1–3 days, along with [^{14}C]-microscale standards (Amersham). The developed films were scanned with a flatbed transmissive scanner (PowerLookII, UMAX) and converted into pseudocolors according to the optical density using the program NIH Image (Version 1.6), running on the Power Macintosh 9500. For emulsion coating, the glass slides were defatted by the serial exposure to ethanol and chloroform. Thereafter, sections were dipped in Kodak NTB-2 (diluted 1:1 with double distilled water), dried overnight at RT, and stored in light-tight boxes at 4 °C for 7–10 days. After development (Kodak D-19 and Kodak Rapid Fixer), the sections were lightly counter-stained with methyl green (Vector Laboratory Inc., Burlingame, CA). The slides were then dehydrated in graded ethanol, cleared in Americlear and coversliped by Permount. Immunohistochemistry was used to assay brain sections for neuronal localization. To this end, free-floating sections were washed with PBS, blocked with 3% horse serum and 0.25% Triton X-100 in PBS containing 120 U/ml rRNasin (Promega) for 1-h, and incubated overnight at 4 °C with a mouse anti-neuronal nuclei antibody (NeuN, Chemicon) at a dilution 1:500 in PBS containing 120 U/ml rRNasin. Following brief rinsing in PBS, sections were incubated with biotinylated horse anti-mouse IgG (1:250, Vector) for 2 h, rinsed and processed with an avidin–biotin complex (Vectastine Elite ABC kit, Vector), and developed by 3,3′-diaminobenzidine (DAB, Sigma).

3. Results

In the past, various albino (non-pigmented) and pigmented rat strains were examined for differences in their pineal glands. It was found that the size and the melatonin-synthesizing capacity of the pineal gland are the greatest in the albino rats, whereas the smallest

pineals were found in the totally pigmented Brown–Norway rats (Vollrath et al., 1989). Using quantitative RT-PCR with internal standards to assay the brain (i.e. hippocampal) content of AANAT mRNA, we also compared the pigmented Brown–Norway (BN) rats with two albino strains: Sprague–Dawley and Fisher 344. The results of this assay underwent two types of analysis—(a) as absolute amounts of AANAT mRNA (attomoles) per total RNA content (μg); and (b) as absolute amounts of AANAT mRNA corrected by the values of corresponding absolute amounts of cyclophilin (cyc) mRNA, and expressed as AANAT/cyc ratios (Fig. 1). We found AANAT mRNA in the hippocampal samples of all the three rat strains we investigated. Although the absolute amounts of this mRNA were rather small (hundreds of attomoles vs. a thousand attomoles of the cyc mRNA, or thousands of attomoles of pineal AANAT mRNA), both of the above-noted types of analysis revealed that hippocampal AANAT mRNA content in the pigmented rats was almost twice the amount we found in either of the two albino rat strains (Fig. 1). Therefore, the pigmented BN rat strain was used in most of our further experiments. In order to verify that the signal we amplify by our quantitative RT-PCR assay (oligonucleotides were designed to allow amplification of 51–493 bp of AANAT; Coon et al., 1995) is indeed AANAT mRNA, the RT-PCR product obtained from the hippocampal tissue was sequenced and its identity was confirmed.

We investigated the localization of the RT-PCR-amplified products (AANAT mRNA) using the method of in-situ hybridization we developed specifically for this project (see Section 2). Fig. 2 shows the four areas of the rat CNS in which the AANAT mRNA signal was the most prominent: hippocampus, olfactory bulb, cerebellum, and spinal cord. In these regions, AANAT mRNA was heterogeneously expressed. It was found in the granular layer of the hippocampal dentate gyrus and in the granule neurons of the olfactory bulb and the cerebellum. In the spinal cord sections, we observed the AANAT mRNA signal mostly in the gray matter. Fig. 3 shows the examples of a neuronal localization of the AANAT mRNA in the granular layer of the dentate gyrus of the hippocampus (co-localization with the neuronal marker NeuN), and in the cerebellar granule neurons of the cerebellum. As expected, an in-situ RT-PCR assay of AANAT mRNA in the pineal gland revealed a dramatic difference between daytime and nighttime samples, which suggests an increase in the pineal AANAT mRNA content during the night (Fig. 4).

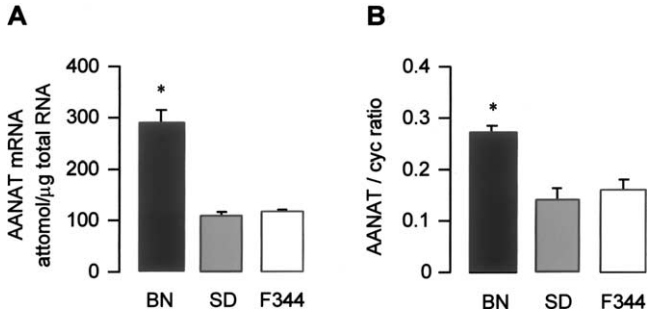

Fig. 1. Strain differences in the content of AANAT mRNA in the rat hippocampus. Adult (2 months) male rats (Brown–Norway, BN; Sprague–Dawley, SD; Fisher 344, F344) were sacrificed between 10:00 and 11:00 h. mRNA content was analyzed by the quantitative RT-PCR assays of AANAT and cyc mRNAs. AANAT mRNA content was calculated per μg total RNA (A) and as AANAT/cyc ratio (B). Both analyzes indicate that BN rats express higher amounts of AANAT mRNA ($n = 5$ per group; mean ± S.E.M.; $P < 0.01$ compared with SD and F344 rats).

We also investigated whether the content of the brain's (e.g. hippocampal) AANAT mRNA is affected by the diurnal rhythm, and found the nocturnal AANAT mRNA up-regulation in the pineal gland but not in the hippocampus (Fig. 5A). It is commonly believed that pineal functioning and melatonin synthesis decrease with age (Reiter, 1995; Waldhauser et al., 1998). Thus, we compared pineal and hippocampal AANAT mRNA contents between 2- and 24-month-old rats. In both tissues, the AANAT mRNA content was lower in old than in young rats (Fig. 5B).

4. Discussion

Our data on the neuronal expression of AANAT mRNA are consistent with the hypothesis that this enzyme may have a physiological function in mammalian CNS neurons (Hamada et al., 1999; Psarakis et al., 1982; Pulido et al., 1983b; Stefulj et al., 2001; Uz and Manev, 1999). In insects such as *Drosophila*, *N*-acetyltransferases are considered as playing a role analogous to the monoamine oxidases (MAO) of mammalian tissues in the inactivation of biogenic amines; MAO enzymatic activity is virtually absent in the fruit fly (Dewhurst et al., 1972; Sekeris and Karlson, 1966). In *Drosophila*, significant AANAT mRNA expression has been found in the nervous system (Hintermann et al., 1996), and melatonin synthesis was demonstrated in fly head homogenates (Finocchiaro et al., 1988). It is questionable whether in the rat, and possibly in mammalian CNS neurons in general, AANAT will lead to significant synthesis of melatonin. Instead, it could rather possibly be the enzyme responsible for the formation of neuronal NAS from serotonin. Namely, no evidence (Ribelayga et al., 1998) or low abundance (Stefulj et al., 2001) for the CNS expression of hydroxyindole-*O*-methyltransferase, the enzyme involved in the last step of melatonin synthesis from NAS, was found in rats, whereas evidence of neuronal NAS localization/production in the rat brain (Chae et al., 1999; Pulido et al., 1983a,b) was reported even before

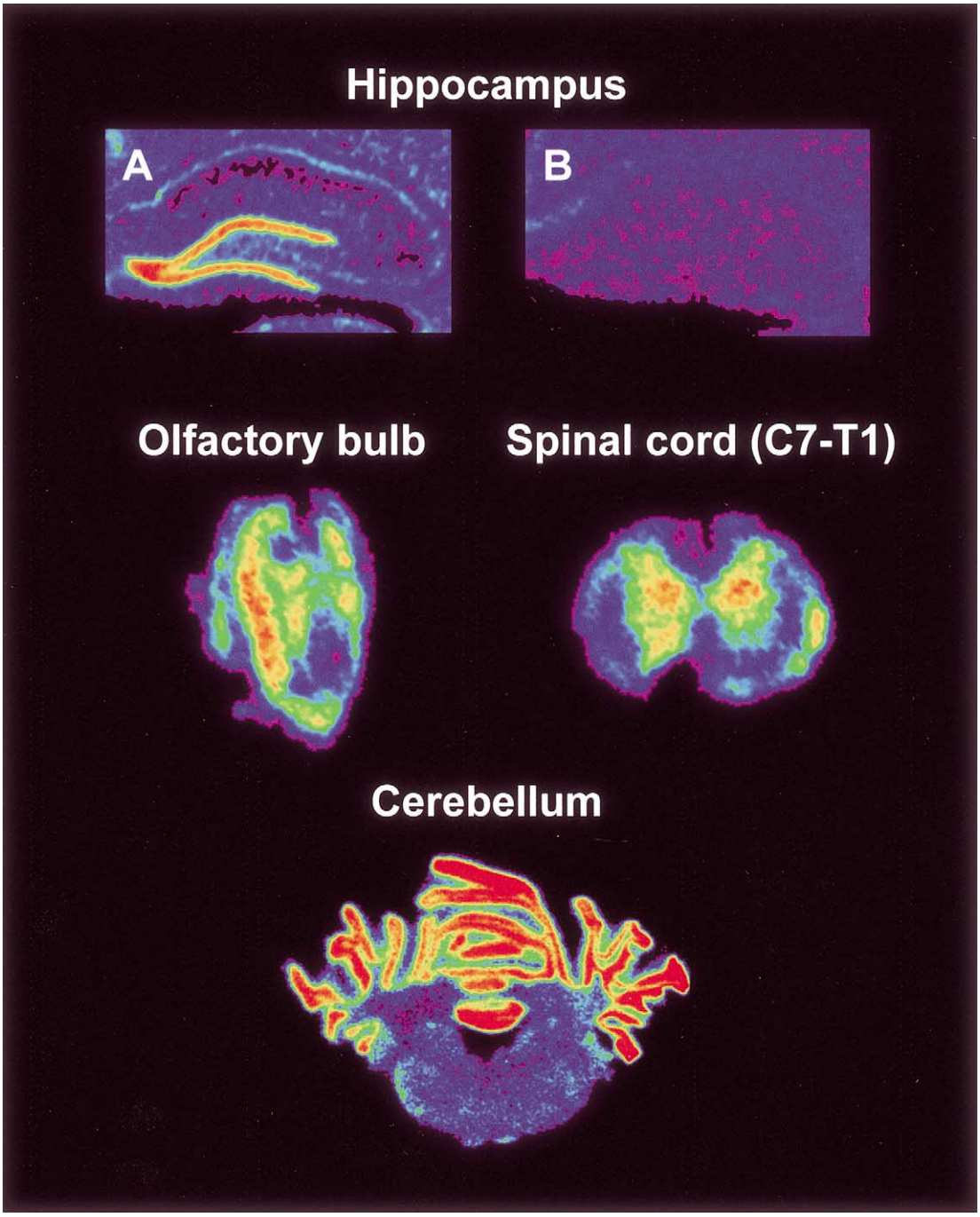

Fig. 2. RT-PCR in situ hybridization assay of AANAT mRNA localization in the rat CNS. The sections (obtained from a 2-month-old, male, Brown–Norway rat) were exposed overnight to the Amersham β-max film. The images were scanned and are presented in pseudocolors (yellow to red indicates the AANAT mRNA signal). Note the presence of AANAT mRNA in the dentate granule cells of the hippocampus (A), in the granule cells of the olfactory bulb, in the gray matter of the spinal cord, and in the cerebellar granule neurons. Panel B shows the absence of AANAT mRNA signal in the hippocampus in conditions in which the Taq DNA polymerase was omitted (negative control). Similar absence of the specific AANAT mRNA signal was obtained by omitting the specific primers from the assay solutions or by 2-h pre-digestion of tissue sections with RNase before RT (not shown).

the tools needed for direct localization of AANAT became available. Alternative sites of extrapineal AANAT activity may include the bone marrow cells (Tan et al., 1999).

The localization of AANAT mRNA that we observed is, to a great extent, in agreement with previous reports on the localization of NAS immunoreactivity in the rat CNS. In particular, our RT-PCR in-situ localization of AANAT mRNA, its co-localization with the neuronal marker NeuN, and the previously-used immunocytochemical detection of NAS (Psarakis et al., 1982; Pulido et al., 1983a), identified cell bodies in the granular layer

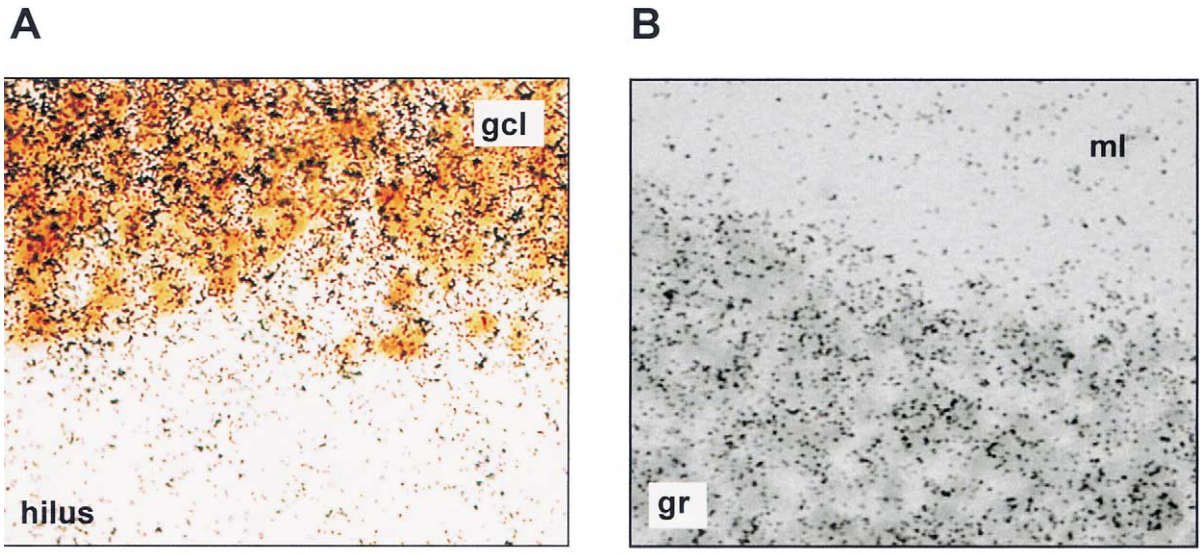

Fig. 3

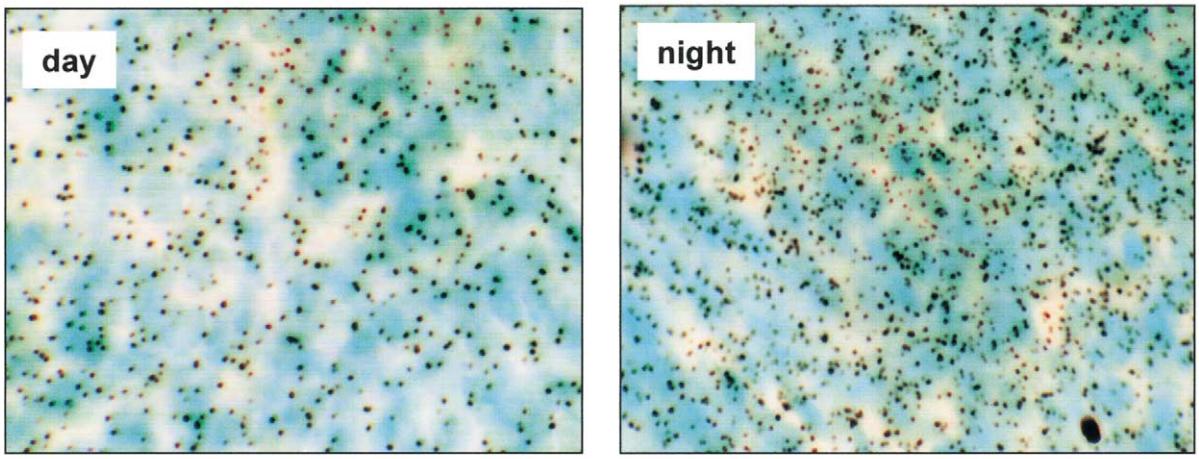

Fig. 4

Fig. 3. The high magnification (40 ×) of the localization of the AANAT mRNA signal in the hippocampus (A) and the cerebellum (B). The photographs were taken from the emulsion-covered hippocampal section co-labeled for the neuronal marker NeuN (brown staining; A) or the emulsion-covered cerebellar section counter-stained with methyl green (B). The black dots indicate the presence of AANAT mRNA; note its localization over neuronal cell bodies. (gcl = granular cell layer; ml = molecular layer; gr = granular layer).

Fig. 4. Effect of diurnal rhythm on pineal AANAT mRNA: RT-PCR in situ hybridization assay. Rats were sacrificed either between 10:30 and 11:00 h (day), or under red light, between 00:30 and 01:00 h (night). The sections were emulsion-covered; the black dots indicate the presence of AANAT mRNA. Note the substantial increase of the AANAT signal in the nighttime sample.

of the hippocampal dentate gyrus and cerebellar granule neurons as sites of possible neuronal NAS synthesis.

In this and in our previous studies (Uz and Manev, 1998) we have observed diurnal variation in pineal AANAT mRNA content. Using a Northern blot assay of pineal and retinal AANAT mRNAs, researchers found dramatic differences between the day- and the night-time samples (Roseboom et al., 1996); however, in these studies, the day-time content of AANAT mRNA was almost undetectable. Our RT-PCR assay is more sensitive; it measures the day-time pineal AANAT and it still detects a major nocturnal mRNA increase. The day-time content of pineal AANAT mRNA was only about ten times greater than the content we found in the hippocampus (Fig. 5A). However, as opposed to the pineal, the hippocampal AANAT mRNA was not affected by the diurnal rhythm. The reason for this discrepancy could lie in the nature of the pathways recruited in the activation of the cAMP system in the pineal versus the hippocampus. Whereas the nocturnal cAMP increase, and the consequent activation of AANAT, are well documented in the pineal gland (Li et al., 1998) no circadian rhythms in cAMP concentration have been found in the hippocampus (Kafka et al., 1986).

The decreases in pineal and hippocampal AANAT mRNA in old (24-month-old) rats that we observed might be a reflection of a general aging-associated

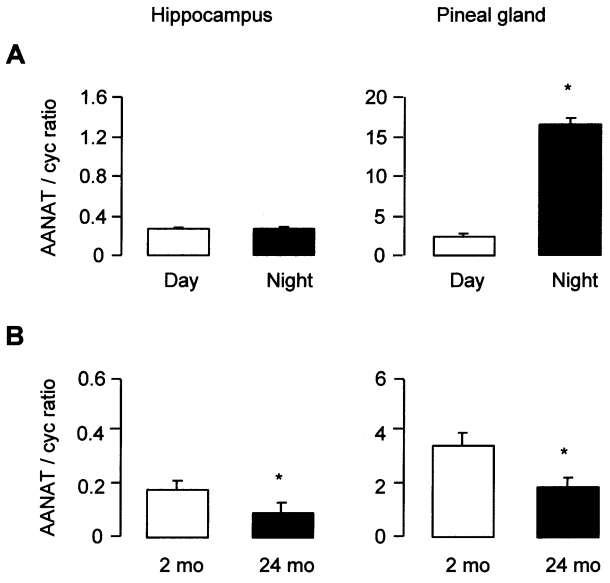

Fig. 5. Effects of diurnal rhythm (A) and aging (B) on hippocampal and pineal content of AANAT mRNA—quantitative assay. Rats (2-month-old) were sacrificed between 10:30 and 11:30 h (day); night-time samples were obtained by sacrificing rats under red light, between 00:30 and 01:00 h. The groups of 2- or 24-month-old rats were sacrificed during the day. The absolute amounts of both AANAT and cyc mRNAs were measured (attomol/μg total RNA) and the corresponding AANAT/cyc ratios were calculated ($n = 5$ per group; mean ± S.E.M.; $^*P < 0.01$, A; $^*P < 0.05$, B).

decrease in the cAMP response element (CRE) binding activity (Asanuma et al., 1996). Such decreases may reduce the basal or the stimulated expression of those genes, such as AANAT (Baler et al., 1997) or the brain-derived neurotrophic factor (BDNF), whose promoters contain the CRE. For example, both basal and stimulated BDNF mRNA expression were significantly decreased in the brains of old rats (Croll et al., 1998; Smith and Cizza, 1996). It is unlikely that aging leads to a nonspecific and general decrease in mRNA expression in the CNS; namely, we observed earlier that hippocampal expression of the mRNA encoding 5-lipoxygenase is greater in 24-month-old than in 2-month-old rats (Uz et al., 1998).

Several possible functional implications of our findings can be envisioned. For example, neuronal AANAT mRNA, if functional, i.e. if it results in functionally relevant amounts of AANAT protein, might decrease local concentrations of serotonin (5-HT) and thereby alter the signaling via this neurotransmitter system. Furthermore, by synthesizing NAS, AANAT may give rise to a biologically active molecule that could either interfere with 5-HT receptors (Niles et al., 1982) or melatonin receptors (Molinari et al., 1996), or might bind to its own, as yet unidentified, binding sites. Finally, it has been proposed that NAS possesses significant antioxidative properties, which also could play a physiological role, e.g. in neuroprotection (Longoni et al., 1997).

As yet, only scarce data about the functional activity of NAS are available. For example, when NAS was administered to mice intraperitoneally, and its action was assayed by the behavioral 'tail suspension test', it was found that NAS decreased the immobility in this test, an indication of its possible antidepressant-like action (Prakhie and Oxenkrug, 1998). In line with this finding is also our recent observation on the effect of the antidepressant fluoxetine on the hippocampal AANAT mRNA content in rats. Since it has been proposed that chronic antidepressant treatment might be effective because it up-regulates the expression of the cAMP response element binding protein (CREB), and, as a consequence, also stimulates the expression of the hippocampal CRE-regulated genes, we investigated the effect of such treatment on hippocampal AANAT mRNA. We found that chronic (21 days) but not the acute administration of fluoxetine to rats resulted in about a fivefold increase in hippocampal AANAT mRNA (Uz and Manev, 1999). Thus, both the antidepressant-like action of NAS (Prakhie and Oxenkrug, 1998), and the up-regulation of hippocampal AANAT by antidepressants (Uz and Manev, 1999), suggest that one of the possible roles of neuronal AANAT might be involvement in the pathobiology of depression and/or in the mechanisms of action of antidepressant drugs. These latter mechanisms are still poorly understood and may involve a variety of biological processes, including cell proliferation in the adult brain (Manev et al., 2001). Further studies are warranted of the possible functional significance of the neuronal expression of AANAT.

Acknowledgements

This work was supported in part by NIH grants RO1-AG15347 and RO1-MH061572 (H. Manev).

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