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Angiotensin receptor II is present in dopaminergic cell line of rat substantia nigra and it is down regulated by aminochrome

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Abstract

Angiotensin receptor II mRNA was found to be expressed in dopaminergic neuronal cell line RCSN3 of rat substantia nigra using RT-PCR reaction. Aminochrome (150 μM), a metabolite of the dopamine oxidative pathway, was found to down regulate the expression of angiotensin receptor mRNA in RCSN3 cells by 83% ($p < 0.05$). (Mol Cell Biochem **212**: 131–134, 2000)

Key words: dopamine, aminochrome, angiotensin receptor II, substantia nigra, quinone

Introduction

The existence of a renin-angiotensin system in mammalian brain is supported by several investigations [1–3]. Angiotensin II (AGT II) receptors subtypes and their distribution in the central nervous system (CNS) have been reported [4–7]. There is evidence supporting the idea that AGT II is involved in cognitive processes. It has been shown that intracerebroventricular administration of AGT II facilitates the acquisition in active conditioning trial and retention in passive avoidance models [8–11], improving recognition, but not spatial memory in rats [12]. The facilitatory effect of AGT II on memory appears to be mediated, in part, by the dopaminergic system since it is abolished by pimozide, a dopaminergic receptor antagonist [13]. In agreement, a bilateral 6-OH-dopamine induced lesion of the dopaminergic projection from A10 ventral tegmental neurons to the central amygdala abolished the facilitatory effects of AGT II [14, 15] and recognition memory [16].

Dopamine has been reported to be oxidized enzymatically and nonenzymatically to *o*-quinone aminochrome [17–24]. The one-electron reduction of aminochrome to a leukoaminochrome *o*-semiquinone radical has been suggested to be responsible for the degeneration of dopaminergic system in Parkinson Disease [25, 26]. We have speculated that the AGT II system may be involved in the loss of memory in Parkinson's patients with dementia, therefore we decided to study the expression of AGT II receptor mRNA in RCSN-3 cells, to investigate a possible modulation by aminochrome of regulation of AGT II receptor. For comparison, the effect of aminochrome on the expression of DT-diaphorase mRNA was studied.

Materials and methods

Chemicals

Dopamine, was purchased from Sigma Chemical Co. (St Louis, MO, USA). Thermoscript RT-PCR system and Taq DNA polymerase were from Life Technologies (California,

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USA). RNeasy midi system was from QIAGEN (Hilden, Germany). Aminochrome was prepared by oxidizing dopamine with Manganese^{3+}-pyrophosphate complex according to Segura-Aguilar and Lind [18].

Cell culture

A RCSN-3 cell line was derived from the substantia nigra of a 4 month old Fisher 344 normal rat. The cell material used to establish primary cultures was transformed to a permanent cell line by exposing them to media conditioned by UCHT1 cells, a process that induces transformation in cell cultures [27]. The RCSN-3 cell line grows on monolayers, with a doubling time of 52 h, a plating efficiency of 21% and a saturation density of 410.000 cells/cm^2. RCSN-3 cells possess receptors for tetanus toxin, and immunohistochemical analysis has demonstrated the presence of neuronal markers such as microtubular associated protein-2 (MAP-2), neuronal specific enolase (NSE), parvalbumin, and tyrosine hydroxylase. Conversely, glial markers glial fibrillary acidic protein (GFAP) and S-100 have not been observed [28]. Also RCSN-3 cells exhibited intracellular fluorescence with paraformaldehyde-glioxilate and are positive for melanin staining, indicating the presence of catecholamines [28]. The cultures were kept in an incubator at 37°C with 100% humidity and an atmosphere of 10% CO_2. The cells were treated with 150 μM aminochrome.

Expression of AGT II receptor

The expression of AGT II receptor in a RCSN-3 cell line was studied by using the RT-PCR technique. The total RNA was isolated by using RNeasy Midi kit (QIAGEN). Five μg of the total RNA was used for the synthesis of a single strand DNA with the reverse transcriptase (RT) reaction. The RT-reaction was performed by using a Thermoscript RT-PCR system (Life Technologies) with Oligo $(dT)_{20}$ as primers. The amplification of ssDNA of AGT II was performed by PCR reaction using the following primers 5′- GATGCTGGTAGCCAAAGTCACC-3′ (upstream) and 5′- GATAAGGAAAGGGAACACGAAGC-3′ (downstream) designed from the cDNA sequence of AGT II receptor [29]. The PCR reaction was performed in three steps: (i) 95°C for 5 min; (ii) 20, 25–30, 35 and 40 cycles at 95°C for 40 sec, 65°C for 40 sec, 72°C for 40 sec; (iii) one cycle at 72°C for 10 min. The PCR incubation contained 6 ml of RT-incubation, 0.4 mM dNTP each, 3 mM $MgCl_2$, 2,5 μM primers, 5 μl 10 × PCR-buffer (GibcoBRL), 29 ml H_2O and 2 U Taq polymerase (GibcoBRL). The region amplified by PCR was between the bases 561–582 and 768–746, which resulted in a fragment of 208 bp [29]. PCR at different number of cycles was performed to estimate the differences in the level of expression of AGT II receptor mRNA between control and aminochrome-treated RCSN-3 cells. The PCR products were electrophoresed on 2% agarose gels, stained with ethidium bromide and photographed. The photographs were scanned and computer analyzed by using a Scion Image software (NIH, USA) in order to estimate the differences in the expression of AGT II mRNA. The number of pixels estimated by the software was plotted against the number of cycles. DT-diaphorase was used as control protein to demonstrate that the aminochrome effect on AGT II mRNA expression was specific.

Expression of DT-diaphorase

The PCR reaction for DT-diaphorase was performed as described by Arriagada *et al*. [30] using the following primers 5′-CAGAAACGACATCACAGGGGAG-3′ (upstream) and 5′-CAAGCACTCTCTCAAACCAGCC-3′ (downstream). The region amplified by PCR was between the bases 230 and 438, which resulted in a fragment of 209 bp [30].

Results

AGT II receptor mRNA was constitutively expressed in a dopaminergic neuronal cell line RCSN-3 of rat substantia nigra as assessed by RT-PCR technique. The effect of aminochrome on the expression of AGT II receptor was studied by

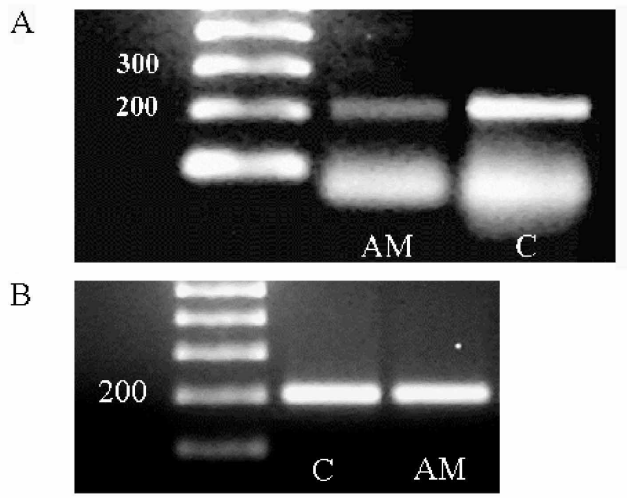

Fig. 1. Expression of angiotensin II receptor mRNA in RCSN3 cells of rat substantia nigra and the effect of aminochrome on expression of its mRNA. The expression of AGT II receptor (A) and DT-diaphorase (B) mRNA was measured using RT-PCR technique described under Materials and methods. The cells were incubated in the presence of 150 μM aminochrome (AM) during 2 h before extraction of total RNA. The AGT II band is observed at 208 kb. The control (C) cells were incubated under the same conditions in the absence of aminochrome.

incubating the cells with 150 μM of aminochrome during 2 h before extraction of the total RNA. Aminochrome was found to down regulate the expression of AGT II mRNA in RCSN3 cells (Fig. 1A). The specificity of this down regulation of AGT II mRNA by aminochrome was supported by the lack of effect of aminochrome on DT-diaphorase mRNA expression (Fig. 1B). PCR reactions at different cycle numbers was performed to estimate the down regulation of AGT II receptor exerted by aminochrome (Fig. 2). The down regulation was assessed as 83% at 40 cycles ($p < 0.05$).

Discussion

The brain renin-AGT II system has been suggested to exert facilitatory effect on memory [17], which appears to be mediated, in part, by the dopaminergic systems [18–20]. The finding that AGT II receptors are present in dopaminergic neurons of substantia nigra and that aminochrome down regulates the expression of AGT II mRNA receptor raises a question on the role of aminochrome in the regulation of brain AGT II receptor. Aminochrome can be formed *in vivo* by oxidation of dopamine by oxygen, transitions metals (iron, manganese or iodine) [17–20] and peroxynitrite radical [21]. Dopamine is also oxidized to aminochrome by several enzymes, such as prostaglandin H synthase, xanthine oxidase, several forms of cytochrome P450 and, specially, by CYP 1A2 [22–24]. The possible formation of aminochrome *in vivo* is supported by the finding that cysteinyl adducts, such as 5-cysteinyl-dopamine and quinone adducts, are present in rat, guinea pig and human brain [31–33]. In addition, aminochrome is the precursor of neuromelanin. The reductive metabolism of aminochrome to leukoaminochrome *o*-semiquinone has been proposed to be the reaction responsible for the degenerative process characterizing Parkinson's disease [25, 26]. Leucoaminochrome *o*-semiquinone is very reactive with oxygen, resulting in the reduction of dioxygen to superoxide radicals. Superoxide radicals, enzymatically or non-enzymatically, dismutate resulting in the formation of hydrogen peroxide and dioxygen. Hydrogen peroxide is the precursor of one of the most harmful free radicals, hydroxyl radical (·OH), in the presence of metals such as Fe^{2+} and Cu^{+}. Furthermore, leukoaminochrome *o*-semiquinone is a radical that can react by itself with nucleophile molecules, such as RNA, DNA, GSH, or induce lipid peroxidation and deactivation of enzymes by oxidation of essential thiol groups. Paradoxically, the antioxidant enzymes, superoxide dismutase and catalase, have been reported to play a prooxidante role during one-electron reduction of aminochrome by increasing the autoxidation rate of leukoaminochrome *o*-semiquinone radical [25]. However, whether AGT II receptor down regulation observed by aminochrome may in part explain the loss of memory in Parkinson's patients with dementia remains to be further investigated.

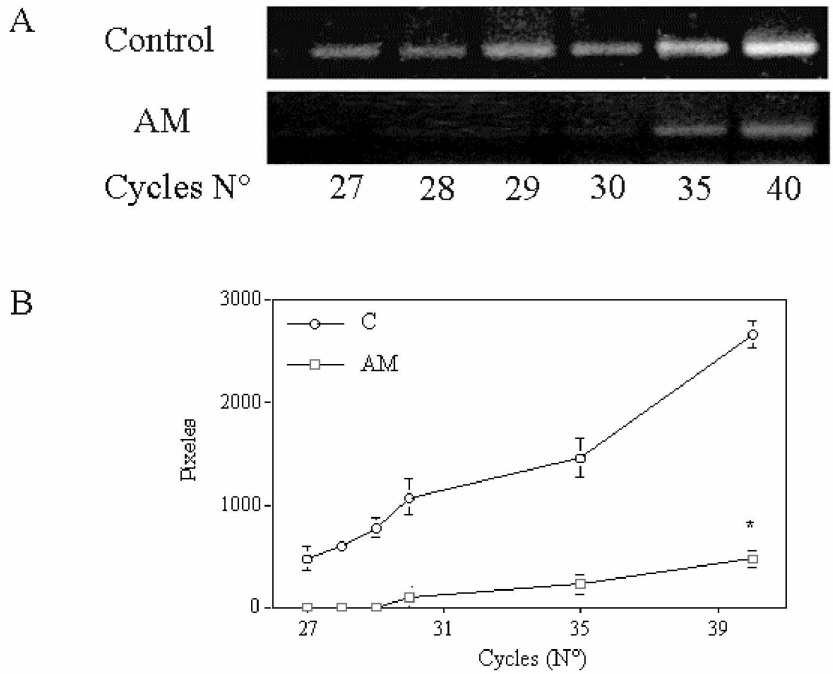

Fig. 2. Estimation of down regulation of AGT II receptor mRNA by using PCR. (A) PCR reactions at different cycle numbers in the presence and absence of 150 μM aminochrome. (B) Estimation of differences in the expression of AGT II mRNA was performed by scanning the gel showed in A. The values are the mean ± S.D. ($n = 3$). $p < 0.05$ vs. control was determined by using the unpaired Student's *t*-test.

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