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Short communication

Effects of single and repeated stresses on the expression of mRNA for α_1-adrenoceptors in the rat hypothalamus and midbrain

Satoru Miyahara [a,*], Teruhisa Komori [a], Ryoichi Fujiwara [b], Koji Shizuya [a], Masato Yamamoto [a], Masaki Ohmori [b], Yuji Okazaki [a]

[a] *Department of Psychiatry, Mie University School of Medicine, Edobashi 2-174, Tsu, Mie 514-8507, Japan*
[b] *First Department of Pathology, Kagawa Medical School, Kita, Kagawa 761-0701, Japan*

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Abstract

We examined the effects of single or repeated stress on the expression of mRNA for α_1-adrenoceptors in the rat hypothalamus and midbrain using the reverse transcriptase-polymerase chain reaction (RT-PCR). Single stress significantly increased the mRNA level for α_1-adrenoceptors in the midbrain, but had no effect on mRNA levels in the hypothalamus. Repeated stress significantly decreased mRNA levels for α_1-adrenoceptors in both regions.

Keywords: α_1-Adrenoceptor; Stress; α-Adrenoceptor mRNA; Hypothalamus; Midbrain

1. Introduction

It is known that various responses to stress are related to changes in sensitivity of adrenoceptors. Among the subtypes of adrenergic receptors, activation of α_1-adrenoceptors may play an important role in stressful conditions. α_1-Adrenoceptors are widely distributed in the rat brain (Young and Kuhar, 1980) and a number of investigators have demonstrated a relationship between stress and central α_1-adrenoceptors, especially in the hypothalamus (Al-Damluji, 1993). It is reported that catecholamines stimulated the hypothalamic–pituitary–adrenal axis (Plotsky et al., 1989) via α_1-adrenoceptors in the hypothalamic paraventricular nuclei, which might mediate the stimulatory effects from the ascending catecholaminergic pathways (Whitnall, 1993). Although the sensitivity of α_1-adrenoceptors has been examined using a variety of methods, including measurement of immunoactivity (Williams and Morilak, 1997) or α_1-adrenoceptors binding (Perters, 1984), the effects of stress on levels of α_1-adrenoceptor mRNA in the rat brain have not yet been examined.

The present study was conducted to determine whether single or repeated stresses influence the level of α_1-adrenoceptor mRNA in the rat hypothalamus and midbrain, using reverse transcriptase-polymerase chain reaction (RT-PCR).

2. Experimental procedure

Twelve-week-old male Wistar rats were purchased from SLC (Shizuoka, Japan) and used in this study. They were housed in cages placed in a quiet room with controlled light/dark cycle (lights on from 6:00 a.m. to 6:00 p.m.) and temperature (22 ± 2°C). Rats were individually exposed to restraint stress by wrapping in a wire gauze in the prone position for 4 h (9:00 a.m. to 1:00 p.m.) (single stress; $n = 6$) or 4 h per day (9:00 a.m. to 1:00 p.m.) for three consecutive days (repeated stress; $n = 6$). Control rats ($n = 6$) were not restrained. Rats were decapitated immediately after exposure to restraint stress. The brains were removed and the hypothalamus and midbrain were rapidly dissected out as described by Glowinski and Iversen (1966). The tissue samples were stored at -80°C until analysis. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Mie University Medical School.

* Corresponding author. Tel.: +81-59-231-5018; fax: +81-59-231-5208

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Total RNA was extracted from the hypothalamus and midbrain as described by Chomczynski and Sacchi (1987). The hypothalamus included supraoptic nuclei and paraventricular nuclei that are predominantly innervated with noradrenergic and adrenergic terminals by various cell groups and seem to contain part of the hypothalamic–pituitary–adrenal axis (Plotsky et al., 1989). The midbrain included the midbrain, thalamus, and subthalamus, which contain catecholamine neuron terminals (Brownstein and Palkovits, 1984), but are not directly associated with the hypothalamic–pituitary–adrenal axis. Tissues were lysed in the presence of guanidinium thiocyanate and phenol. The RNA was extracted with chloroform and isopropanol. It was precipitated twice in ethanol and then dissolved in diethylpyrocarbonate-treated water. Levels of RNA were determined spectrophotometrically. The total RNA sample from each region of the brain was used for RT-PCR. Levels of α_1-adrenoceptor mRNA and glyceraldehyde-3-phosphate dehydrogenase mRNA were determined by semiquantitative RT-PCR. Reverse transcription was performed with a First-Strand cDNA Synthesis Kit (Pharmacia Biotech, Tokyo, Japan) according to the levels recommended by the manufacturer. The reaction mixture was incubated at 37°C for 60 min. Primers for amplification of cDNA for α_1-adrenoceptors were synthesized by Takara Shuzo (Shiga, Japan) and were as follows: sense primer, 5′-CCCAATGATGACAAAGAATG-3′; and antisense primer, 5′-TGAAGTAGCCCAGCCAGAAC-3′. Primers for the cDNA for glyceraldehyde-3-phosphate dehydrogenase (sense primer, 5′-ACCACAGTCCATGCCATCAC-3′; and antisense primer, 5′-TCCACCACCCTGTTGCTGTA-3′) were obtained from Clontech (Palo Alto, CA). The predicted sizes of amplified products were 454 bp for cDNA of α_1-adrenoceptors and 452 bp for cDNA of glyceraldehyde-3-phosphate dehydrogenase. To normalize signals from different samples of RNA, mRNA for glyceraldehyde-3-phosphate dehydrogenase was reverse transcribed and cDNA was amplified as an internal standard. Amplification reactions with both sets of oligonucleotide primers were stopped before the end of the exponential phase (α_1-adrenoceptors, 27 cycles; glyceraldehyde-3-phosphate dehydrogenase, 21 cycles). After reverse transcription, 20 μl of each reaction mixture was heated at 95°C for 5 min to inactivate the reverse transcriptase and subjected to PCR with specific primers for α_1-adrenoceptors and glyceraldehyde-3-phosphate dehydrogenase in separate tubes. Each total reaction mixture for PCR contained 10 mM Tris–HCl (pH 8.3), 20 mM KCl, 2.5 mM $MgCl_2$, 0.02 mg/ml bovine serum albumin, 0.5 mM each dNTP, 20 pmol sense and antisense primers, 2.5 U Taq DNA polymerase and 5 μCi [α-^{32}P]dCTP. The conditions for each cycle of PCR amplification of cDNA of α_1-adrenoceptors and glyceraldehyde-3-phosphate dehydrogenase were 94°C for 1 min, 55°C for 40 s and 72°C for 1 min. PCR products were separated on a 3.5% polyacrylamide gel and detected by electric autoradiography (InstantImager; Packard, TOWN, CT). The radioactivity of each band of cDNA for α_1-adrenoceptors was compared to that of cDNA of glyceraldehyde-3-phosphate dehydrogenase and the result was expressed relative to the control value. Data for α_1-adrenoceptors mRNA were normalized by reference to the data for glyceraldehyde-3-phosphate dehydrogenase mRNA, determined from the same sample, and the mean ± S.E.M. was calculated from six replicate amplifications. Each experiment was repeated twice. Statistical analysis was performed using analysis of variance (ANOVA) and Fisher's test. Statistical significance was recognized when $P < 0.01$.

3. Results

A representative result, showing the products of analysis by RT-PCR, after staining with ethidium bromide, of α_1-adrenoceptor and glyceraldehyde-3-phosphate dehydrogenase mRNAs in the hypothalamus, is shown in Fig. 1. The intensities of the bands varied according to the type of stress (single vs. repeated). The results of semiquantitative analysis of these bands are shown in Fig. 2. The radioactivity of cDNAs for α_1-adrenoceptors in the hypothalamus and midbrain was corrected by reference to the radioactivity of cDNA amplified from the constitutively expressed mRNA for glyceraldehyde-3-phosphate dehydrogenase. When rats were exposed to a single 4-h episode of restraint

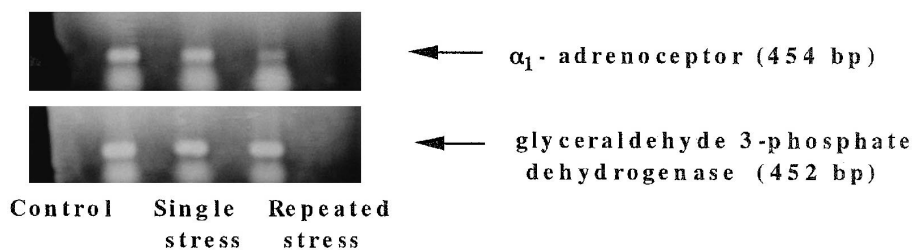

Fig. 1. Results of staining cDNAs of α_1-adrenoceptors and glyceraldehyde-3-phosphate dehydrogenase with ethidium bromide. The intensity of the band reflects the level of the corresponding mRNA in the rat hypothalamus.

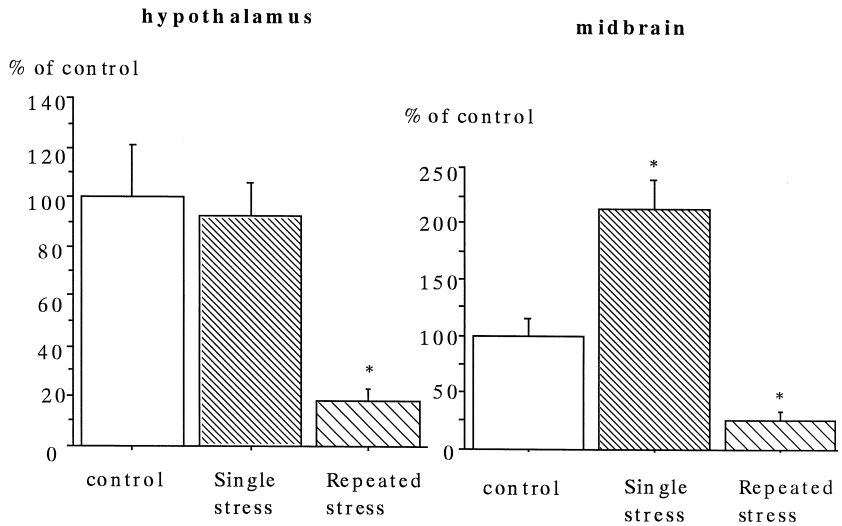

Fig. 2. mRNA levels of α_1-adrenoceptors in the rat hypothalamus and midbrain after single and repeated stress and in control rats. Data were normalized by reference to results of glyceraldehyde-3-phosphate dehydrogenase mRNA and are expressed as means ± S.E.M. for six rats. $^*P < 0.01$ vs. control.

stress, the level of mRNA for α_1-adrenoceptors was not significantly affected in the hypothalamus (Fig. 2). In contrast, 3 days of repeated restraint stress (4 h/day), resulted in a significant decrease in the level of α_1-adrenoceptor mRNA in this region. In the midbrain, the level of α_1-adrenoceptor mRNA increased significantly after a single restraint stress, but decreased significantly after repeated stress (Fig. 2).

4. Discussion

To our knowledge, there are no studies that have previously examined changes in α_1-adrenoceptors mRNA in various rat brain regions following exposure to stress. Our results indicated that repeated episodes of stress, but not one single stress, significantly reduced α_1-adrenoceptors mRNA expression in the hypothalamus. Our results also indicated that the response of the midbrain was different, significant increases in mRNA expression in this region were noted after a single stress; whereas repeated stress resulted in a significant fall.

Cizza et al. (1995) used microdialysis to investigate changes in noradrenaline levels in the extracellular fluid of paraventricular nuclei of the hypothalamus before and during a single stress (immobilization for 2 h) in rats. They reported that stress significantly increased the concentration of noradrenaline. On the other hand, Singh et al. (1988) used a radioenzymatic procedure to investigate changes in noradrenaline and adrenaline levels in the cortex, hypothalamus and thalamus following chronic electric shock stress. They reported that chronic stress did not alter the concentrations of noradrenaline and adrenaline in the hypothalamus and thalamus. It is possible that the negative results in the above study could have been due to adaptation to chronic stress. Other investigators have examined the response of α_1-adrenoceptors to acute stress (Gibson et al., 1986; Kiss and Aguilera, 1992). The results of these studies suggested that activation of the hypothalamic–pituitary–adrenal axis in response to various stresses might depend on the activation of ascending noradrenergic pathways, particularly stimulation of central α_1-adrenoceptors in the hypothalamus (Gibson et al., 1986; Kiss and Aguilera, 1992). Recently, Takita et al. (1997) showed that chronic stress caused no significant change in affinity for [^{3}H]prazosin or in the maximum number of α_1-adrenoceptor sites in the rat cerebral cortex.

In the present study, single stress resulted in overexpression of α_1-adrenoceptor mRNA in the midbrain, but did not change the level of the same mRNA in the hypothalamus. Although we cannot explain the mechanism of regional differences in the mRNA response, it is likely that activation of α_1-adrenoceptors might occur simultaneously with increased release of noradrenaline in the rat brain, resulting in increased α_1-adrenoceptor mRNA in the midbrain. In the hypothalamus, activation of α_1-adrenoceptors might have resulted in immediate activation of the hypothalamic–pituitary–adrenal axis, but the level of α_1-adrenoceptor mRNA did not change due to a feedback by hypothalamic–pituitary–adrenal axis, although the sensitivity of α_1-adrenoceptor might be enhanced by single stress. In fact, previous studies have shown that the plasma concentration of adrenocorticotropic hormone (ACTH) reached a maximum level within 15 min of immobilization of rats for 120 min (Takaki et al., 1994).

Our study also showed that repeated stress resulted in a significant reduction of mRNA levels of α_1-adrenoceptors in both regions of the brain. The exact mechanism of the observed fall in mRNA is not clear at present and to our knowledge, no previous studies have demonstrated a reduction of the mRNA of α_1-adrenoceptors in the hypothalamus and midbrain during repeated stress. It is possible

that the need for α_1-adrenoceptors might have decreased during repeated stress. Further studies are needed to clarify the significance of the present results by investigating the sensitivities and number of α_1-adrenoceptors during various stresses.

5. Conclusion

We have demonstrated in the present study, using a method based on RT-PCR, that stress produces changes in α_1-adrenoceptor mRNA levels in different regions of the brain. Our novel findings indicated that the pattern of mRNA changes varied from one brain region to another in response to single stress; increased mRNA levels in the midbrain, but no change in the hypothalamus. However, this differential effect was not observed for repeated stress; both regions showed a fall in mRNA levels.

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