Agonist-induced down-regulation of α₁B-adrenergic receptor in HEK293 cells transfected with α₁B cDNA

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Received December 5, 1997

Abstract HEK293 cells stably expressing hamster α₁B-adrenergic receptor (α₁B-AR) were used to observe the effect of norepinephrine (NE) on α₁B-AR gene expression. Radioligand binding assays and RNase protection assays were used to determine α₁B-AR number and the mRNA level, respectively. Exposure (2—24 h) of HEK293 cells to NE (10 μmol) caused a decrease in α₁B-AR mRNA with maximum change found at the 4th hour, and in α₁B-AR density at the 24th hour. NE-induced decrease in α₁B-AR mRNA was inhibited by protein kinase C (PKC) inhibitor calphostin C (0.1 μmol) and mimicked by PKC activator PMA (1 μmol). Nuclear run-off transcription assay showed that treatment of the cells with NE (10 μmol) exerted no effect on the transcription rate of α₁B-AR. After the synthesis of new RNAs was inhibited by actinomycin D, NE could not accelerate the degradation of α₁B-AR mRNA. The results suggested that in the HEK293 cells NE could induce the down-regulation of α₁B-AR, and the effects were mediated by PKC pathway. NE could not alter the transcription rate of α₁B-AR mRNA, but it might induce the synthesis of some factors and indirectly accelerate the degradation.

Keywords: α₁B-adrenergic receptor, desensitization, down-regulation, gene expression.

α₁-adrenergic receptors (α₁-AR) belong to the superfamily of G-protein-coupled receptors. Prolonged activation of these receptors would lead to a decrease in agonist sensitivity. The process may be related to receptor down-regulation, sequestration and phosphorylation, etc. Desensitization of α₁B-AR induced by agonists has been reported, but the results remained controversial. Lurie et al. [1] reported that in the presence of agonist α₁B-AR-mediated contraction response was reduced in rabbit aorta although the number of α₁B-AR was unchanged. But Izzo et al. [2] found that in cultural rabbit aortic smooth muscle cells, prolonged agonist exposure would cause a decrease in the density of α₁B-AR. In contrast, prolonged activation of protein kinase C (PKC) would induce transcription and expression of α₁B-AR gene in DDT₁ MF-2 cells [3]. In the present study, human embryonic kidney cells (HEK293) were transfected with full length hamster α₁B cDNA to obtain the stable cells line expressing α₁B-AR. The transfected cells were used as a model to observe the effects of NE on α₁B-AR expression.

1 Materials and methods

1.1 Cell transfection and culture

The plasmid containing hamster α₁B-AR cDNA (pREP/α₁B) was used to transfected HEK293

* Project supported by the National Natural Science Foundation of China (Grant No. 39470268) and the China Medicine Board of New York Inc.

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cells by calcium phosphate-mediated gene transfer method. The transfected cells were cultured at 37°C in Dubcco's modified Eagle's medium containing 10% newborn bovine serum, 4.5 g/L glucose, 200 000 U/L penicillin and 100 mg/L streptomycin.

1.2 Radio ligand binding assay
Cultured cells were digested with 0.08% trypsin, washed once with phosphate buffer saline (PBS), then suspended in 20 mL PBS, homogenized with a polytron homogenizer. The pellet was obtained by centrifugation for 10 min at 20 000 g, finally resuspended in adequate volume of PBS for later use. Total binding was determined by incubating 100 μL of membrane suspension with 13-500 pmol/L 125I-BE2254 (125IBE) in a 250 μL volume of 50 mmol/L Tris-HCl buffer containing 1% BSA. Nonspecific binding was determined in the presence of 10 μmol/L phentolamine. After 20 min incubation at 37°C, 10 mL ice-cold buffer was added to each tube and immediately filtered through a Whatmann GF/B fiberglass filter under vacuum suction. The retained radioactivity was measured by a γ-counter. Receptor affinity (K_D values) was measured by saturation experiments and total cellular receptor density (B_max values) was measured by scatchard plots.

1.3 RNA extraction
A single-step method[41] of RNA isolation using acid quanum thiocyanate phenol/chloroform extraction was used to isolate total RNA from the cultured HEK293 cells. RNA samples were quantified using a spectrophotometer at 260/280 nmol/L and then aliquoted into 20 μg. Samples were stored at −70°C for later use.

1.4 RNase protection assay
A 668-bp fragment of the full length hamster a1B cDNA was ligated into the Bam H1/Xhol site of pBSK(-). A 819-bp fragment of human β-actin cDNA was subcloned into pst1 site of pGEM-1 and used as an internal control. Linearized cDNA constructs were used to make antisense radiolabeled RNA probes (specific radioactivity≈ 1.5 × 10^6–2.0 × 10^8) using T3 or SP6 RNA polymerase as described in the Promega Guide Manual. Probes were labeled with [α-32P]UTP. Total RNA (20 μg) was used in RNase protection assay as described previously[5]. The autoradiographic bands for a1B and β-actin were quantified by imaging analysis system (Leica Q550IW, Germany). a1B mRNA levels were expressed as ratios of signal of a1B bands to that of β-actin.

1.5 Nuclear run-off transcription assay
Cells were exposed to NE for 4 h, then harvested. The cell pellet (2 × 10^7) was resuspended in 2 mL NP40 lysis buffer (10 mmol/L Tris-HCl pH7.0, 10 mmol/L NaCl, 3 mmol/L MgCl2, 0.5% (v/v) NP40), incubated for 5 min on ice and centrifuged at 500 g for 5 min. The supernatant was removed. The nuclear pellet was washed once with 2 mL lysis buffer and centrifuged at 500 g. After the supernatant was discarded, the remaining nuclei were resuspended in 200 μL storage buffer (50 mmol/L Tris-HCl pH8.3, 40% (v/v) glycerol, 5 mmol/L MgCl2, 0.1 mmol/L EDTA) and immediately mixed with 200 μL reaction buffer (10 mmol/L Tris-HCl pH8.0, 5 mmol/L MgCl2, 300 mmol/L KCl, 0.5 mmol/L each of CTP, GTP and ATP, and 100 μCi of [α-32P]UTP). The mixture was incubated at 30°C for 30 min. The labeled RNA was extracted with phenol, phenol/chloroform and chloroform, finally precipitated by ethanol. This labeled RNA was dissolved in 2 mL hybridization buffer (the same to that in RNase protection as-