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Short communication

Nipradilol inhibits DNA synthesis by regulating nitric oxide synthesis in cultured rat mesangial cells

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Abstract

To elucidate whether nipradilol modulates mesangial cell function, we assessed the effects of nipradilol on DNA synthesis in the presence and absence of *N*-nitro-L-arginine methyl ester (L-NAME) in cultured rat mesangial cells stimulated with 1% fetal bovine serum. Nipradilol inhibited [^{3}H]thymidine incorporation into mesangial cells in a dose- and time-dependent manner. In addition, the anti-mitogenic effect of 100 μM nipradilol was significantly inhibited in the presence of 10 μM L-NAME. Moreover, nipradilol increased intracellular cyclic guanosine monophosphate (cGMP). These results suggest that nipradilol exerts its efficacy in the treatment of several types of glomerulonephritis with mesangial cell proliferation by increasing in intracellular cGMP.

Keywords: Nipradilol; Nitric oxide (NO); DNA synthesis; Glomerular mesangial cell

1. Introduction

Glomerular mesangial cell proliferation is considered one of the major histological characteristics of various glomerular diseases. It is thought to be stimulated by inflammatory events and to participate in the interactive process that leads to glomerulonephritis (Shultz and Raiji, 1991). Therefore, pharmacological intervention in mesangial cell proliferation may be important in the treatment of various glomerular diseases. Recently, in addition to a physiological role, a pathophysiological role has been proposed for nitric oxide (NO) in mesangial cells. These cells produce large amounts of NO in response to cytokines (Shultz et al., 1991), and this could lead to the mesangial cell dysfunction seen in glomerular diseases (Cattell and Cook, 1993).

Nipradilol (3,4-dihydro-8-(2-hydroxy-3-isopropylamino)proxy-3-nitroxy-2H-1-benzopyran) is a nonselective adrenoreceptor blocking agent with weak β-adrenoreceptor blocking activity and direct vasodilating activity (Uchida et al., 1983). Since nipradilol contains an NO_2 group within its molecular structure, it may release NO and affect mesangial cell function. The present study was conducted to assess whether nipradilol modulates DNA synthesis in cultured rat mesangial cells stimulated with serum in the presence and absence of a NO synthesis inhibitor, *N*-nitro-L-arginine methyl ester (L-NAME).

2. Materials and methods

2.1. Mesangial cell culture

Kidneys were harvested from 6–8-week old male Wistar rats as previously described (Eto et al., 1997). Glomeruli were isolated by differential sieving, digested with 0.1% collagenase, cloned and cultured in RPMI 1640 medium containing 15% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 μg/ml insulin. Cultured cells were identified as mesangial cells by their morphological characteristics and positive staining for vimentin, desmin, and Thy 1.1 antigen. Cells at passages 4–9 were used for the experiments after reaching confluence.

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2.2. Cell proliferation assays

Thymidine incorporation was measured as previously described (Nitta et al., 1995a). Mesangial cells were transferred to 24-well plates, 10^4 cells per well, allowed to rest for 48 h in serum-free RPMI medium, and then stimulated for 22 h with 0.01 to 100 μM nipradilol in the presence or absence of 10 μM L-NAME dissolved in the medium. The stimulated cells were then pulsed with 0.5 mCi/ml [^{3}H]thymidine for 2 h, harvested on glass-fiber paper, and scintillation were counted. In some experiments, cells were trypsinized and counted with a hemocytometer.

2.3. cGMP measurement

After the final incubation period, monolayers were washed three times with phosphate-buffered saline, and the cellular cGMP was extracted with sodium acetate buffer, pH 6.2. The cGMP content of the cells was measured by radioimmunoassay using a commercially available kit. ^{125}I-cGMP tracer was diluted to provide 7000 counts/min per 100 μl, and antibody was diluted to provide maximum binding of 40–50% of total counts. Samples were acetylated before assay, and antigen–antibody complexes were precipitated with excess rabbit nonimmune immunoglobulin G and polyethylene glycol. Recovery of cGMP by this method was $>95\%$.

2.4. LDH release

To examine the cytotoxic effect of nipradilol, the supernatants from monolayers incubated with the agents were tested for lactate dehydrogenase (LDH) release by spectrophotometric assay with a commercial kit, as previously described (Nitta et al., 1995b).

2.5. Chemicals

Nipradilol was kindly provided by Kowa Pharmaceutical, Nagoya, Japan. [^{3}H]thymidine was purchased from Amersham, Tokyo, Japan, and a cGMP assay kit from New England Nuclear, Washington, USA. FBS and RPMI medium were from Gibco BRL, New York, USA, and L-NAME and insulin were from Sigma, St. Louis, USA. All other reagents were of chemical grade and purchased from standard suppliers.

2.6. Statistical analysis

Results are expressed as means ± S.D. Comparisons between two groups were made using Student's unpaired *t*-test. Comparisons among three or more groups were performed by one-way analysis of variance (ANOVA) followed by Bonferroni's test to evaluate statistical significance between any two groups. Data were considered statistically significant if $P < 0.05$.

3. Results

The effect of nipradilol on [^{3}H]thymidine incorporation into mesangial cells stimulated with 1% fetal bovine serum is shown in Fig. 1a. At concentrations of 10 μM and 100 μM, nipradilol significantly inhibited [^{3}H]thymidine incorporation (10 μM; 19.5%, 100 μM; 60%). Fig. 1b shows the time course of [^{3}H]thymidine incorporation during incubation with 100 μM nipradilol. After 16-h and 24-h incubation, nipradilol had significantly inhibited [^{3}H]thymidine incorporation into mesangial cells stimulated with 1% fetal bovine serum. Nipradilol did not alter the morphologic appearance of the cells, or LDH release by the mesangial cells (data not shown), suggesting that

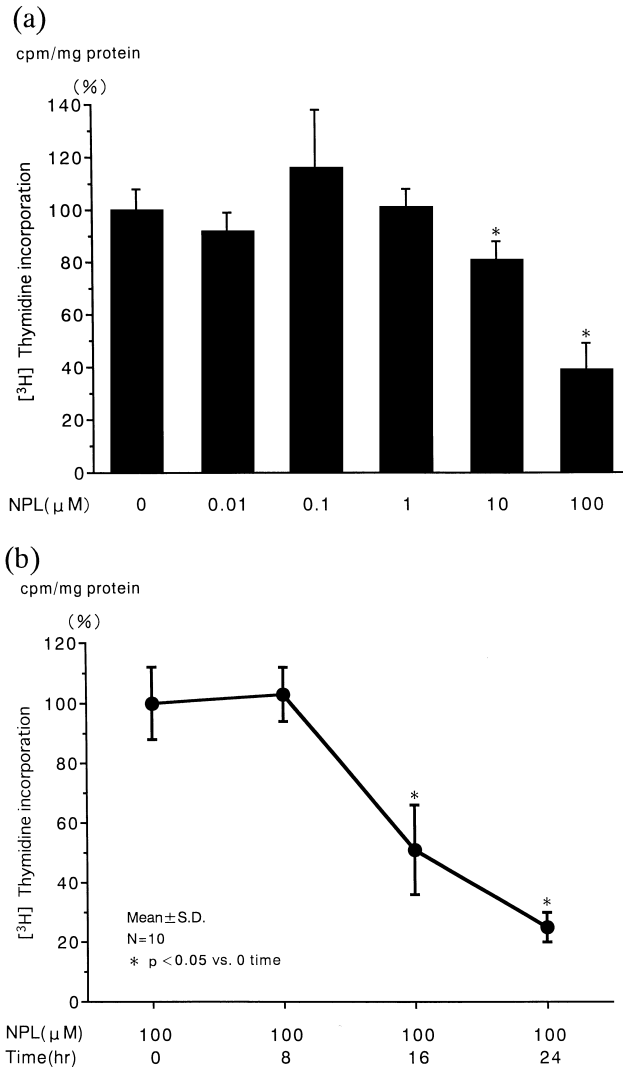

Fig. 1. (a) Effect of nipradilol (NPL) on serum-induced [^{3}H]thymidine incorporation in cultured rat mesangial cells. Cells were treated with various doses of nipradilol for 24 h in the presence of 1% fetal bovine serum (FBS). Values are means ± S.D. ($n = 6$). * $P < 0.05$ vs. NPL (−). (b) Time-course of the effect of 100 μM nipradilol on serum-induced [^{3}H]thymidine incorporation in cultured rat mesangial cells. Cells were incubated with 100 μM nipradilol in the presence of 1% FBS for the times indicated. Values are means ± S.D. ($n = 10$). * $P < 0.05$ vs. time 0.

toxicity could be ruled out as a cause of the inhibition of [^{3}H]thymidine incorporation.

To investigate the mechanism of the anti-mitogenic effect of nipradilol, we assessed the effect of L-NAME, a NO synthesis inhibitor. As shown in Fig. 2a, 10 μM L-NAME significantly blocked the anti-mitogenic effect of nipradilol on the mesangial cells stimulated with 1% fetal bovine serum ($p < 0.05$, for nipradilol vs. nipradilol plus L-NAME). No significant toxicity of these doses of nipradilol or L-NAME was detected on the basis of cell counts, phase-contrast morphology, or measurements of LDH released by mesangial cells (data not shown).

In an attempt to identify a potential second messenger for the inhibitory effects of nipradilol on mesangial cells, intracellular levels of cGMP were measured after incubating them with nipradilol. A significant increase in intracellular cGMP levels was detected when incubated for 10 min with 100 μM nipradilol in the presence of 1% fetal bovine serum (Fig. 2b, vs. 1% FBS only), and 10 μM L-NAME significantly inhibited intracellular cGMP levels in cells incubated for 10 min with 100 μM nipradilol in the presence of 1% FBS.

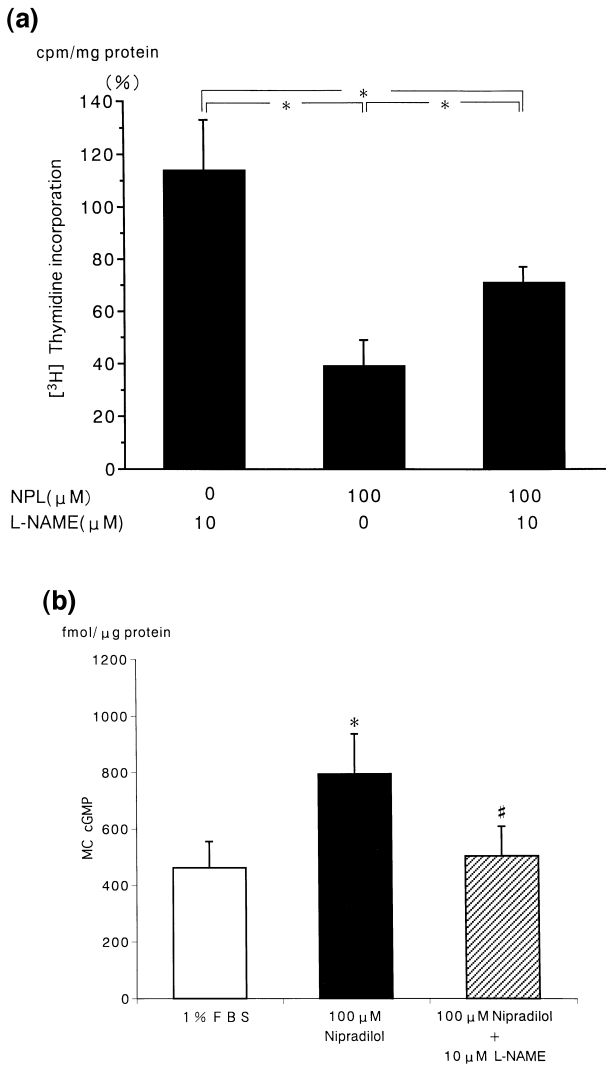

Fig. 2. (a) Effect of L-NAME on nipradilol-induced [^{3}H]thymidine incorporation in cultured rat mesangial cells. Cells were treated with 1% FBS and 100 μM nipradilol for 24 h in the presence and absence of 10 μM L-NAME. Values are means ± S.D. ($n = 6$). $*$ $P < 0.05$ vs. L-NAME (+). (b) Effect of L-NAME on nipradilol-induced intracellular cGMP levels in cultured rat mesangial cells. Cells were exposed to 1% FBS and 100 μM nipradilol in the presence and absence of 10 μM L-NAME at 37°C for 10 min. Values are means ± S.D., $n = 6$. $*$ $P < 0.05$ vs. 1% FBS, $\#P < 0.05$ vs. Nipradilol.

4. Discussion

The results of the present study show that nipradilol, a nonselective β-adrenoreceptor blocking agent, inhibits DNA synthesis in cultured rat glomerular mesangial cells. This effect of nipradilol was correlated with the increase in intracellular cGMP levels. NO-generating vasodilators such as sodium nitroprusside and isosorbide dinitrate have been reported to increase intracellular cGMP and inhibit mesangial cell proliferation (Garg and Hassid, 1989). Since nipradilol contains an NO_2 group within its molecular structure, it is thought to induce an antimitogenic effect on mesangial cells similar to sodium nitroprusside and isosorbide dinitrate.

Although the effective dose of nipradilol required to inhibit DNA synthesis (10 μM to 100 μM) appears to be higher than its effective plasma concentrations as an antihypertensive drug, pharmacokinetic studies in rats have indicated that the concentrations of nipradilol in the kidney are almost 50 times higher than that in the blood after single oral administration of nipradilol (Kimata et al., 1985). Local concentrations of nipradilol might reach sufficient levels to increase intracellular cGMP, followed by inhibition of DNA synthesis in mesangial cells.

Glomerular mesangial cells play an important role in regulating the glomerular filtration rate, and their proliferation is one of the most important pathogenic mechanisms of glomerular sclerosis (Grandaliano et al., 1993). Our results show that nipradilol can inhibit DNA synthesis in mesangial cells through an increase in intracellular cGMP levels that results in NO production. Therefore, it is suggested that nipradilol has a beneficial effect in the treatment of various types of glomerulonephritis with mesangial cell proliferation.

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