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Neuropeptide Y Stimulates Prostacyclin Production in Porcine Vascular Endothelial Cells

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We investigated the effects of neuropeptide Y on the prostacyclin production of cultured porcine aortic endothelial cells by measuring the stable metabolite of prostacyclin, 6-keto-prostaglandin $F_{1\alpha}$, by radioimmunoassay. Neuropeptide Y induced dose- and time-dependent stimulation of prostacyclin production by cultured porcine aortic endothelial cells. The lowest stimulatory concentration of neuropeptide Y was 10^{-8} M and maximal response, a 2.8 fold rise, was obtained with 10^{-6} M. The stimulation lasted at least 24 h. The effect was associated with the stimulation of arachidonic acid release. Our data suggest that neuropeptide Y may inhibit the development of atherosclerosis by stimulating prostacyclin synthesis.

Prostacyclin(PGI_2), a major arachidonic acid metabolite of vascular endothelial cells, is a powerful vasodilator and a potent inhibitor of platelet aggregation(1, 2). Thus, PGI_2 may be an important antithrombotic factor. Despite the importance of PGI_2, little is known about the regulation of its production.

In addition to adrenergic and cholinergic nerve fibers, immunohistochemical and radioimmunoassay studies have revealed nerve fibers containing neuropeptides, such as neuropeptide Y in the arteries(3, 4). However, the relationship between neuropeptide Y and PGI_2 synthesis has not been studied.

In the present report the effect of neuropeptide Y on PGI_2 synthesis by porcine aortic endothelial cells was investigated.

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Abbreviations: NPY, neuropeptide Y; PGI_2, prostacyclin; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$.

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MATERIALS AND METHODS

Isolation and culture of porcine endothelial cells: Porcine endothelial cells were isolated by scraping the intimal surface of isolated thoratic aorta with a surgical blade and were suspended in Eagle's minimum essential medium(MEM)(Sigma, St. Louis, MO, USA) supplemented with 20 % fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin. Primary cultures were grown in 75-cm^2 culture flasks(Corning) maintained at 37°C in 5% CO_2 in air. The medium was changed 12-24 h after seeding and thereafter every second or third day. Only those cultures were used which grew to confluency in less than 7 days.

The confluent cells in 75-cm^2 flasks were detached with 0.2% EDTA and 0.5% trypsin(Sigma, St. Louis, MO., USA) and seeded in 24-well plates(Corning). All culture flasks and plates were pretreated overnight with collagen type I(Sigma). Cultured endothelial cells were characterized by polygonal cell morphology and strict contact inhibition of cell growth. Cells from passages 2-4 were used in this series of experiments.

Staining with fluorescent Di-I-Ac-LDL: The cells were treated with the fluorescent probe acetylated low density lipoprotein(Di-I-Ac-LDL). The cells were incubated in the presence of 10 µg/ml of Di-I-Ac-LDL for 4 hours at 37°C, after which time the media was removed and the cells were washed three times with PBS. The LDL particles were visualized by fluorescence microscopy with filters adjusted for rhodamine fluorescence.

Effect of NPY on PGI_2 production: The effect of NPY on PGI_2 production was studied in 24-well plates using confluent monolayers of endothelial cells. Immediately before each experiment the culture medium was aspirated from the confluent monolayers and these were washed once with culture medium. Fresh culture medium(1.0 ml) was added without(control) or with 10^{-8} - 10^{-5} M porcine NPY(Sigma) and incubated for 1 - 24 h at 37°C in 5 % CO_2 in air.

Release of labeled arachidonic acid from endothelial cells: The [^{14}C]arachidonic acid-labeled cells were prepared by incubating stationary phase endothelial cells with medium containing [^{14}C]arachidonic acid(0.05 µCi/ml per well) in the 24-well plates for 18 h. After labeling, the medium was removed and the cell layer was quickly washed three times with 1 ml of serum-free medium. The labeled cells were then exposed to 1 ml of culture medium without(control) or with 10^{-6} M NPY for 3 h. After 3 h incubation, the medium from each well was collected in tubes and centrifuged at 2000 rpm for 10 min to remove any contamination of free cells or cell debris. 0.5 ml of the supernatant was carefully transferred to a counting vial and the radioactivity was measured by a liquid scintillation counter.

Statistical Analysis: Student's t-test was used to determine the statistical significance. All results are expressed as means $\pm$ S.D..

RESULTS

Cellular characterization

When incubated in the presence of Di-I-Ac-LDL, the cells from the porcine aortic explants demonstrated fluorescent deposits throughout the cytoplasm(Fig. 1).

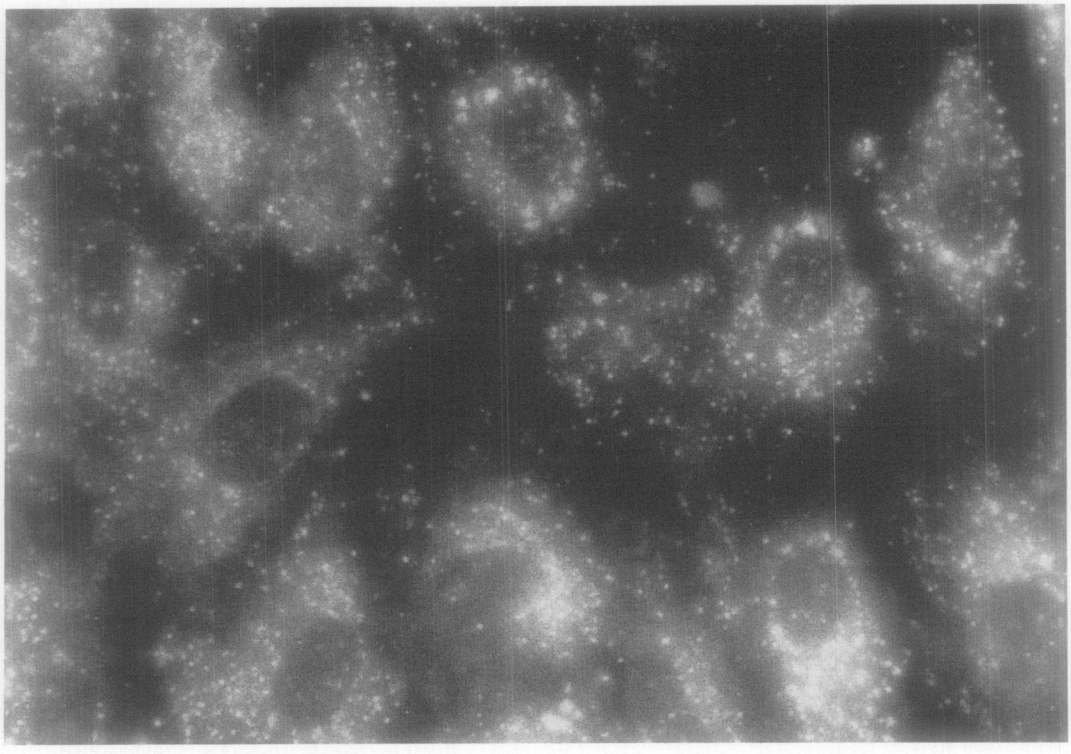

Fig. 1. Labeling of endothelial cells with DiI-Ac-LDL. The cells were incubated with 10 μg/ml of DiI-Ac-LDL for 4 hours at 37°C. The cells were visualized using a standard rhodamine excitation: emission filter set.

Effects of NPY on prostacyclin production

NPY increased the release of 6-keto-$PGF_{1\alpha}$ in a dose dependent manner. The lowest stimulatory concentration was 10^{-8}M and the concentration giving the maximal(2.8 ± 0.5 fold) response was 10^{-6} M(Fig. 2). The effect of NPY was evident after incubation for 2 h and increased progressively between 2 and 24 h(Fig. 3).

Effect of NPY on arachidonic acid release

After preincubation with [^{14}C]arachidonic acid for 18 h, cells were again cultured with a fresh medium supplemented with NPY for 3 h. More than 98 % of the radioactive arachidonic acid was taken up into the cells within 18 h. As shown in Fig. 4, NPY stimulated the release of [^{14}C]arachidonic acid in a dose-dependent manner. The dose curve of the release of [^{14}C]arachidonic acid is similar to that of the release of prostacyclin as described above(Fig. 2). The results indicate that the stimulatory effect of NPY on prostacyclin production is due to the activation of phospholipase A_2/C.

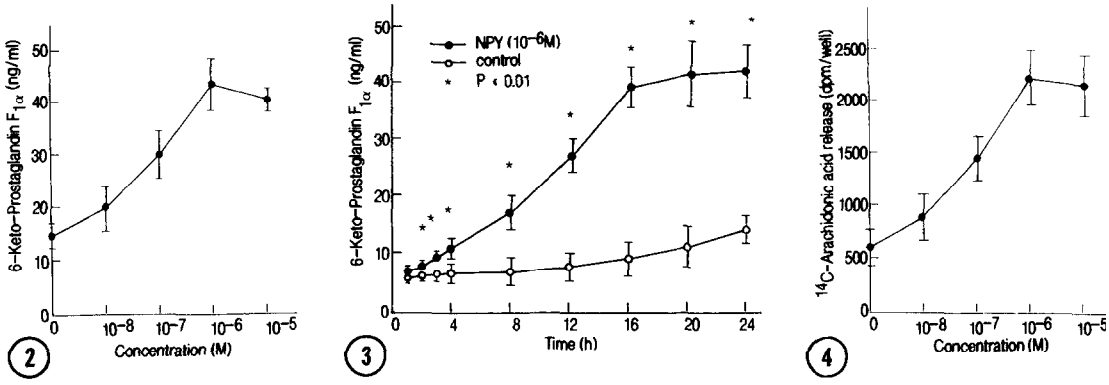

Fig. 2. Effect of NPY on the release of prostacyclin from porcine aortic endothelial cells. Porcine NPY was added to the confluent porcine aortic endothelial cell culture. The amounts of 6-keto-$PGF_{1\alpha}$ in the supernatants were measured by radioimmunoassay after 24 h incubation. Data represent mean ± S.D. of 18 assays from 3 independent experiments.

Fig. 3. Time course of the effect of NPY on prostacyclin production. NPY was added at a concentration of 10^{-6} M to the culture of endothelial cells at indicated times prior to assay for 6-keto-$PGF_{1\alpha}$. Data represent mean ± S.D. of 18 assays from 3 independent experiments.

Fig. 4. Effect of NPY on arachidonic acid release from porcine aortic endothelial cells. After preincubation with [^{14}C]arachidonic acid for 18 h, cells were again cultured with a fresh medium supplemented with NPY(10^{-6} M) for 3 h. Each point represents mean ± S.D. of 18 assays from 3 independent experiments. *P < 0.01 between NPY and control.

DISCUSSION

The effect of NPY of prostacyclin production by porcine aortic endothelial cells was estimated by measuring the levels of its stable derivative, 6-keto-$PGF_{1\alpha}$. The present data are the first demonstration that NPY stimulates the synthesis of prostacyclin in porcine endothelial cells. Alhenc-Gelas and co-workers tested the effects of angiotensin II, vasopressin, substance P, bradykinin, histamine, norepinephrine, and isoproterenol on PG production(5). Of the various vasodilator and vasoconstrictor agents applied to endothelial monolayers, only histamine and bradikinin increased the synthesis of prostaglandins. However, Gerritsen reported that isoproterenol stimulated prostacyclin synthesis(6). The discrepancy between these results is probably caused by different origin of the endothelial cells. Brown and Deykin showed that passage state strikingly and nonuniformly affected prostacyclin release in response to agonist stimulation(7). In our experience, NPY also did not stimulate the synthesis of prostacyclin in

porcine endothelial cells of more than 15 passages. However, we used porcine endothelial cells of 2-4 passages and founded NPY stimulated synthesis of prostacyclin. Thus, under different condition discrepancy may occur.

In addition to adrenergic and cholinergic nerve fibers, immunohistologic and radioimmunoassay studies have revealed nerve fibers containing neuropeptide such as NPY in arteries(3, 4). Edvinsson et al. reported that the number of nerve fibers containing NPY in the cerebral arteries decreased in an age-dependent manner(4). Atherosclerosis is thought to be well correlated with aging(8). Prostacyclin is thought to be an important antithrombotic and antiatherogenic factor(1, 2). Tokunaga et al. reported that prostacyclin synthesis of the aortic endothelial cells decreased with age and they concluded that the decreased synthesis of prostacyclin with age may play an important role in the development and advancement of thrombosis and atherosclerosis(9). From these observations we suggest that NPY may play an important role in antiatherogenesis due to increase production of prostacyclin.

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